RESEARCH 54

Eira Poikonen

Epidemiology of Candidemia in Finland

Academic dissertation

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Candida albicans (green) and *Candida glabrata* on a chromogenic plate

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To my family

Abstract

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Background and aims. *Candida* species are an important cause of nosocomial bloodstream infections (BSIs) in hospitalized patients worldwide, with associated high mortality, excess length of stay and costs. Main contributors to candidemias are intensive treatments, complex gastroenterology surgery, prolonged and profound immunosuppression, invasive monitoring and broad spectrum antibiotic treatments leading to an increasing number of susceptible patients. The rank order of causative *Candida* species varies over time and in different geographic locations. The aim of this study was to obtain information on epidemiology of candidemia in Finland, to identify trends in incidence, causative species, and patient populations at risk as well as the contribution of fluconazole consumption and prophylaxis policies in tertiary care centers (TCCs) to the epidemiology of these infections. In order to reveal possible outbreaks and assess the value of one molecular typing method, restriction enzyme analysis (REA), in epidemiological study, we analyzed *C. albicans* bloodstream isolates in Uusimaa region in Southern Finland during eight years.

Methods. All microbiology laboratories in Finland have reported all bacterial and fungal isolations from blood to the National Infectious Disease Register (NIDR) since 1995. The data from NIDR were used to assess the incidence and epidemiological features of candidemia cases during two separate periods during 1995–1999 and 2004–2007. Data on fluconazole consumption in Finland was obtained from National Agency of Medicines (currently FIMEA). Infectious diseases specialists were interviewed for regional antifungal prophylaxis policies. In Helsinki University Central Hospital (HUCH) all patients with blood culture yielding any *Candida* spp. were identified from laboratory log-books during 1987-1998 and during 1999-2004 from Finnish Hospital Infection Program (SIRO). Patient characteristics and outcome were assessed through chart review and data from this register. Data on fluconazole use were obtained from hospital pharmacy.

During 1986–1995, all patients with a stored blood culture isolate of *C. albicans* were identified through microbiology laboratory logbooks for epidemiologal typing. Place of treatment and patients characteristics were identified through chart review. Stored *C. albicans* isolates were genotyped with REA (using two enzymes) in the National Institute for Health and Welfare (former KTL).

Results. During 1995–1999 the annual incidence of candidemia in Finland increased from 1.7 to 2.2 per 100,000 population. The incidence rose in men 16–65 years of age. *C. albicans* accounted for 70% of cases without a change in proportion.

Compared to the 1990s, during 2004–2007 the average annual incidence rate was higher, 2.9 per 100,000 population, (range by year 2.6–3.1), without increasing trend. The incidence was highest in males >65 years of age, but incidence rates for patients <1–15 years were lower than during 1990s. *C. albicans* accounted for 67% of cases, while *C. glabrata* ranked the second. The crude case-fatality rate in one month varied between 28–32% during 2004–2007. Fluconazole consumption increased in Finland from 19.57–25.09 defined daily doses (DDDs) per 100,000 population during 2000–2007. Fluconazole prophylaxis was routinely used in patients treated for acute leukemias, liver transplant recipients, and in premature infants.

In HUCH the annual incidence varied from 0.26 to 0.59 per 10,000 patient days, without any trend. The most common species was *C. albicans* (65%), while *C. parapsilosis* ranked the second (13%) and *C. glabrata* the third (9%). The proportion of *C. albicans* decreased from 71% to 58%, while the proportion of *C. glabrata* increased from 3% to 14% during observation. Patients treated in intensive care units increased from 27% to 44%, associated with surgical patients and newborns. The one-month case fatality ranged between 30–33%.

REA differentiated efficiently between *C. albicans* blood culture isolates, and demonstrated highly reproducible DNA patterns among consecutive isolates from the same patient. No clusters or endemic subtypes were observed in the seven acute care hospitals involved, despite of abundant transfer of patients among them.

Conclusions. The incidence of candidemia in Finland is globally relatively low, but increased between between 1990s and 2000s. In HUCH the incidence of candidemia remained low and constant, but a significant shift in patient-populations at risk was observed, associated with surgical and intensive care patients. *Candida* spp. are an important cause of nosocomial blood stream infections in Finland, and continued surveillance is necessary to determine the overall trends and patient groups at risk, and reduce the impact of these infections in the future. The predominating causative species in Finland and in HUCH is *C. albicans*, but the proportion of *C. glabrata* increased considerably. The fluconazole consumption increased in all five TCC regions in Finland, and systematic fluconazole prophylaxis was used in traditional high-risk patient groups. The possible role of antifungal prophylaxis with fluconazole in the shift of patient populations at risk and in causative *Candida* spp. should be addressed in the future. The crude case fatality proportion by one month was constantly high. Considering this and the difficulties in administering early and adequate antifungal therapy, better measures of prevention will offer better options to reduce candidemia associated mortality. Epidemiological typing of *C. albicans* did not show any clusters or endemic subtypes in the hospitals involved. Molecular methods provide an efficient tool for investigation of suspected outbreak and should be available in the future in Finland, also.

Keywords: candidemia, invasive candida infections, epidemiology, molecular typing, outcome, *Candida* spp., *C. albicans*

Tiivistelmä

Eira Poikonen. Epidemiology of Candidemia in Finland [Kandidemioiden epidemiologiaa Suomessa]. Terveyden ja hyvinvoinnin laitos (THL), Tutkimus 54/2011. 115 sivua. Helsinki 2011.
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Tausta ja tavoitteet. Kandidahiivat ovat merkittäviä sairaalahoitoon liittyvien veriviljelypositiivisten infektioiden aiheuttajia maailmanlaajuisesti. Kandidemia paitsi pidentää sairaalapotilaiden hoidon kestoa ja siten lisää kustannuksia, myös lisää kuolleisuutta. Kandidemialle ovat alttiita eryisesti potilaat, joiden puolustuskyky infektioille on alentunut vaikean perussairauden, immuunipuolustusta lamaavien hoitojen, tai vaikeiden invasiivisten toimenpiteiden, leikkausten ja monitoroinnin vuoksi. Myös keskosuus ja laajakirjoinen bakteerilääkitys altistavat kandidemioille. Yleisin aiheuttaja maailmanlaajuisesti on *Candida albicans*, mutta pääasiallinen hiivapatogeeni vaihtelee eri aikoina ja eri maissa ja keskuksissa. Vaikkakin suurimmassa osassa kandidemioita aiheuttaja on peräisin potilaan omasta mikrobistosta, on myös sairaalaympäristöstä peräisin olevia epidemioita kuvattu.

Tämän tutkimuksen tarkoituksena oli selvittää kandidahiivojen aiheuttamien veriviljelypositiivisten infektioiden ilmaantuvuutta, aiheuttajalajeja ja kuolleisuutta sairaalapotilailla Suomessa. Lisäksi tutkittiin, mitkä potilasryhmät näitä infektioita sairastavat ja mikä vaikutus hiiva-antibiootti flukonatsolin käytöllä sekä yliopistosairaaloiden flukonatsoliprofylaksikäytännöillä on kandidemioiden ilmaantuvuuteen ja aiheuttajakirjoon. Helsingin-Uudenmaan alueen sairaaloissa hoidetut *C. albicansin* aiheuttamat kandidemiat tutkittiin kahdeksan vuoden ajalta ja laboratorion näistä potilaista tallentamat hiivakannat tutkittiin epidemiologisella tyypitysmenetelmällä.

Menetelmät. Suomen valtakunnallisesta tartuntatautirekisteristä (NIDR) selvitettiin potilaat, joilta veriviljelyssä oli kasvanut kandidahiiva vuosilta 1995–1999 ja 2004–2007. Tartuntatautirekisteriin ilmoitettujen tietojen perusteella voitiin laskea kandidemioiden ilmaantuvuus eri ikäryhmissä ja eri yliopistosairaala-alueilla Suomessa. Tapauksiin liittyvä kuolleisuustieto kuukauden kuluttua infektion toteamisesta oli saatavilla vuosille 2004–2007. Lääkelaitokselta (nykyinen FIMEA) saatiin tiedot flukonatsolin käytöstä yliopistosairaala-alueittain vuosilta 2000–2007. Kunkin viiden yliopistosairaalan infektioerikoislääkäri haastateltiin puhelimitse ja selvitettiin flukonatsolin käyttö sieni-infektioiden estolääkityksenä eri potilasryhmissä sekä lapsi- että aikuispotilailla.

Helsingin Yliopistollisen Keskussairaalan (HYKS) kandidahiivojen aiheuttamat veriviljelypositiiviset infektiot selvitettiin mikrobiologian laboration raporttien perusteella vuosilta 1987–1998 ja valtakunnalliseen sairaalainfektio-ohjelmaan (SIRO) kerättyjen tietojen perusteella vuosilta 1999–2004. Kliiniset tiedot hoitopai-

koista, leikkauksista, ja vierasesineistä kerättiin potilasasiakirjoista vuosilta 1987–1998, vuosilta 1999–2004 käytettiin vain SIRO-rekisteriin kerättyjä tietoja. Lisäksi selvitettiin HYKS:n hallinnosta hoitopäivien määrä tutkimusajalta ja sairaala-apteekista flukonatsolin käyttömäärät vuosilta 1991–1999 ja 2003–2004. Mikrobiologian laboratoriosta selvitettiin otettujen veriviljelyiden määrät ja elinsiirtoja ja luuytimen tai veren kantasolusiirtoja tekevistä yksiköistä tehtyjen elinsiirtojen määrät tutkimusajalta.

Mikrobiologian laboratorion raporttien perusteella poimittiin potilaat, joiden veriviljelyssä oli kasvanut *C. albicans* hiiva vuosina 1986–1995. Tutkimukseen valittiin ne potilaat, joiden hiivakanta oli talletettu ja joita oli hoidettu Helsingin-Uudenmaan alueen sairaaloissa. Potilasasiakirjoista kerättiin kliiniset tiedot ja potilaita hoitavat osastot. Tallennetut *C. albicans* -hiivakannat tutkittiin Terveyden ja Hyvinvoinnin Laitoksen (silloinen KTL) laboratoriossa genotyyppiin perustuvalla tyypitysmenetelmällä, restriktioentsyymianalyysilla (REA).

Tulokset. Kandidemiat lisääntyivät tutkimusaikana: 1995 todettiin 1,7 tapausta/100 000 asukasta ja 1999 2,2 tapausta/100 000 asukasta. Tapaukset lisääntyivät erityisesti 16–65-vuotiailla miehillä. Candida albicans aiheutti 70 % kandidemioista. Vuosien 2004–2007 aikana ilmaantuvuus ei noussut, mutta oli korkeampi kuin 1990-luvulla, keskimäärin 2,9 tapausta/100 000 asukasta (vuosittainen vaihtelu 2,6–3,1/100 000). Ilmaantuvuus 2000-luvulla oli korkein >65-vuotiailla miehillä, mutta nuorilla <1–15-vuotiailla tapauksia oli vähemmän kuin kuin 1995–1999. Pääasiallisin aiheuttaja oli edelleen *C. albicans*, osuus 67 %, mutta *C. glabratan* osuus lisääntyi niin, että 2000-luvulla se oli toiseksi yleisin hiivasepsisten aiheuttaja. Potilaiden kokonaiskuolleisuus kuukauden kuluttua infektion toteamisesta oli 35 % (vuosittainen vaihtelu 32–38 %) vuosina 2004–2007. Flukonatsolin käyttö lisääntyi Suomessa niin, että vuonna 2000 se oli 19,57 DDD (defined daily dose)/100 000 asukasta ja vuonna 2007 25,09 DDD 100 000 asukasta. Flukonatsolia käytettiin rutiinisti lähes jokaisessa yliopistosairaalassa hiivainfektioiden estolääkityksenä akuuttia leukemiaa sairastavilla, maksansiirtopotilailla ja keskosilla.

HYKS:ssä vuosittainen kandidemioiden ilmaantuvuus vaihteli välillä 0,26–0,59 tapausta/10 000 hoitopäivää eikä merkittävää lisääntymistä todettu. Yleisin aiheuttaja oli *C. albicans* (osuus 65 %), toiseksi yleisin oli *C. parapsilosis* (osuus 13 %), ja kolmanneksi yleisin oli *C. gblabrata* (osuus 9 %). *C. albicansin* osuus väheni, sillä vuosina 1987–1992 se aiheutti 71 % tapauksista ja vuosina 1999–2004 58 %, kun taas *C. glabratan* osuus lisääntyi ollen 3 % vuosina 1987–1992 ja 14 % vuosina 1999–2004. Teho-osastoilla hoidettujen ja varsinkin kirurgisten potilaiden osuus lisääntyi, samoin kuin vastasyntyneiden (keskosten) osuus. Potilaista kuoli 30–33 % kuukauden kuluessa infektiosta.

REA erotteli hyvin veriviljelyistä todetut *C. albicans* kannat, ja pystyi toisaalta osoittamaan samasta potilaasta otetuissa toistetuissa näytteissä identtisen kannan. Ryvästymiä tai epidemioita ei todettu huolimatta siitä, että potilaita siirrettiin usein akuuttisairaalasta ja hoitopaikasta toiseen.

Johtopäätökset. Kandidahiivojen aiheuttamien veriviljelypositiivisten infektioiden määrä Suomessa on maailmanlaajuisesti katsottuna pieni, mutta se on lisääntynyt 2000-luvulla 1990-lukuun verrattuna. HYKS:ssä kandidemioiden määrä ei 18 vuoden aikana lisääntynyt, mutta tapauksia ilmaantui seurannan aikana useammin teho-osastoilla hoidetuille potilaille, kuten kirurgisille potilaille ja keskosille. Kandidalajit ovat edelleen Suomessa merkittävä veriljelypositiivisten infektioiden aiheuttaja, joten myös tulevaisuudessa näiden infektioiden ilmaantuvuuden ja aiheuttajalajien seuranta on tärkeää. Kandidemioille alttiit potilaat ja potilasryhmät on pyrittävä tunnistamaan, jotta infektioiden torjuntatoimia tehostamalla voidaan vähentää niiden aiheuttamaa tautitaakkaa ja siten estää niiden aiheuttamaa kuolleisuutta. Siitä huolimatta, että *C. albicans* on edelleen kandidemioiden pääasiallisin aiheuttaja, *C. glabratan* merkitys on lisääntynyt. Flukonatsolin käyttö lisääntyi kaikkien viiden yliopistosairaalan alueella ja sitä käytetään hiivainfektioprofylaksiin perinteisten riskiryhmien potilailla. Flukonatsoliprofylaksin merkitys toisaalta kandidemioille alttiisiin potilasryhmiin ja toisaalta kandidemioiden lajikirjoon voidaan arvioida vasta seurannassa. Potilaiden kokonaiskuolleisuus kuukauden kuluessa infektiosta säilyi vakaasti korkeana. Huomioiden kandidemiaan liittyvä korkea kuolleisuus, diagnoosin vaikeudet ja siten antibioottihoidon riittämättömyys tai viivästyminen, näiden infektioiden ennaltaehkäisy todennäköisesti parantaisi potilaiden ennustetta. Epidemiologinen tutkimus *C. albicansin* aiheuttamista kandidemioista ei osoittanut ryvästymiä tutkituissa sairaaloissa. Molekylaariset tyypitysmenetelmät ovat tarpeen epidemiaselvityksissä ja sellainen tulisi olla kandidoille käytettävissä myös Suomessa.

Asiasanat: Kandidemia, syvä kandidainfektio, epidemiologia, molekulaariset tyypitysmenetelmät, kuolleisuus, Kandidalaji, Candida

Contents

List of original publications

I Poikonen E, Lyytikäinen O, Anttila V, Ruutu P. Candidemia in Finland, 1995–1999. Emerging Infectious Diseases. 2003; 9 (8):985–990.

II Poikonen E, Lyytikäinen O, Anttila V, Ruutu P. Secular trend in candidemia and the use of fluconazole in Finland, 2004–2007. BMC Infectious Diseases. 2010; 10:312.

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Abbreviations

AFLP	Amplified-fragment length polymorphism
AP-PCR	Arbitrarily primed polymerase chain reaction
BSI	Bloodstream infection
CDC	Centers for Disease Control and Prevention (USA)
CVC	Central venous catheter
DDD	Defined daily dose
EK	Electrophoretic karyotyping
FISH	Fluorescent in-situ hybridisation
HCW	Healthcare worker
ICU	Intensive care unit
LOS	Length of stay
MIC	Minimal inhibitory concentration
MICU	Medical intensive care unit
MLST	Multilocus sequence typing
NASBA	Nucleid acid sequence-based amplification
NICU	Neonatal intensive care unit
NIDR	National Infectious Disease Register
NNIS	National Nosocomial Infection Surveillance System (USA)
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PICU	Pediatric intensive care unit
RAPD	Random amplified polymorphic DNA
REA	Restriction enzyme analysis
RFLP	Restriction fragment length polymorphism
SIRO	Finnish hospital infection program
TCC	Tertiary care center
TPN	Total parenteral nutrition

1 Introduction

Bloodstream infections (BSIs) caused by yeasts of the *Candida* genus pose a serious threat to hospitalized patients worldwide. *Candida* species are the fourth leading cause of nosocomial bloodstream infections in the USA, accounting for 7–10% of all BSIs in hospital patients [1–3]. In Europe, *Candida* species ranks 7th to 8th among bloodstream pathogens in hospitalized patients, accounting for 4–6% of BSIs [4, 5]. Candidemias considerably prolong hospitalization and increase the use of healthcare resources—especially costs—as these infections complicate the care of already expensive groups of patients, such as the immunocompromized and critically ill [6–8]. The crude and attributable mortality rates associated with candidemias have remained high, which suggest that prevention of these infections would improve the prognosis of the patients.

Reported incidences of candidemias vary globally. The incidence rates of candidemia considerably increased in the USA during the 1980s and early 1990s [1, 2], while through the 1990–2000s the rates appear to remain stable [2]. The incidence of candidemia also increased during the 1990s in Canada, Australia and Europe, but the reported rates have mostly been lower than in the USA [9–12]. Reasons for the rising candidemia rates include improved detection and, more importantly, an increase in the patient population at risk, as the use of invasive procedures and devices, broad-spectrum antimicrobial agents, advanced life-support and aggressive chemotherapy have become more frequent. The risk for candidemias is high in patients in the extremes of age (preterm neonates and patients >65 years of age) who survive longer with modern treatments. Prior to 1990, candidemias occurred mostly in immunosuppressed patients treated for hematological or solid malignancies, but the incidence of candidemias has more recently increased among non-neutropenic surgical and critically ill patients treated in intensive care units.

The rank order of causative species differs between countries and over time. *C. albicans* is the predominant causative organism, but an increase in the incidence of non-*C. albicans* species has been observed in several centers [13–15], althoug not invariably [16, 17]. The precise distribution of causative species varies across countries and units, for reasons which remain unclear, but may be in part attributable to differences in underlying diseases treated, the types and intensities of treatments and antibiotics used, and other local factors. The emergence of less susceptible *Candida* species is a matter of concern, but the precise role of antifungal drug pressure in promoting the shift towards non-*C. albicans* species remains to be defined.

BSIs caused by *Candida* species lack special clinical findings and fast sensitive diagnostic methods, which can complicate their timely recognition and treatment. To avoid delays in the treatment of candidemia thus worsening the prognosis, prophylactic, empirical, and pre-emptive antifungal treatment strategies have been

developed. But because the identified risk factors for candidemias are all so common among immunocompromised patients and those treated in intensive care units, additional meaningful stratification of these factors is needed to identify the best targets for various treatment strategies [2, 18].

Because the prevalence of risk factors and changes in population demographics predisposing to candidemias are increasing surveillance has become essential. Studies that include selected hospitals or patient populations not only target the defining of risk factors, the distribution of causative species, the antimicrobial susceptibility of the pathogen in question, and its nosocomial spread in a preselected setting, but also allow for observation over prolonged periods of time. Population-based studies, on the other hand, permit the calculations of incidence and mortality rates. The systematic analysis of possible outbreaks, although a minority, is important in defining the mode of transmission and evaluating the control measures and monitoring of infection in special areas where infection is a particular hazard.

The purpose of this study was to investigate the epidemiology of *Candida* bloodstream infections in Finland and particularly in the Helsinki University Central Hospital. With special emphasis on the largest tertiary care center in Finland, we could analyze the impact of molecular typing methods in outbreak surveys, and evaluate the shift in the patient population at risk and in the distribution of causative species over a prolonged period of time. The surveillance data available from National Infectious Diseases Register since 1995 enabled us compare the incidence rates, regional variation and outcome of candidemias during two separate periods in the face of increasing high-risk activities, and thus patient populations at risk for *Candida* blood stream infections.

2 Review of the literature

2.1 The cause and impact of candida infections

2.1.1 Candida species

With more than 200 species that have been described, *Candida* is ubiquitous. Some species are normal habitants of the human microbiological flora of the skin, as well as the gastrointestinal, genitourinary, and even respiratory tracts [19, 20] but only 10% are known to cause infections in humans [21, 22]. Five most common species (i.e., *Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei*) cause 95–97% of human candidemias [2, 3, 23, 24]. The remaining 3–5% of *Candida* bloodstream infections (BSIs) are caused by 12–14 different species, and although these are considered rare causes of candidemias, several have occurred in nosocomial clusters and exhibit innate or acquired resistance to one or more established antifungal agents [20, 24–26].

Main species causing candidemias possess unique properties that by interacting with local factors, may influence the epidemiology of these infections. All five main species are capable of forming biofilms *in vitro*, which confers upon them resistance to antimicrobials and host defence mechanisms [27–29]. Dimorphism and phenotypic switching *in vitro* and *in vivo* may potentiate adherence to and invasion of epithelial cells by *C. albicans* [20, 30]. *C. parapsilosis* can readily colonize the skin and persist in the hospital environment, thus leading to potential nosocomial spread by hand carriage [31, 32]. *C. krusei* is intrinsically resistant to fluconazole and often demonstrates decreased susceptibility to other antifungal agents (i.e., amphotericin B and flucytosine). *C. glabrata,* on the other hand, can easily acquire a resistance to fluconazole and other azole antifungal agents [2, 15].

2.1.2 Sources

The primary origin of candidemia has been the subject of debate, with some suggesting endogenous while others favouring exogenous acquisition. The most important of these is considered as the endogenous acquisition of infection, which is implied when candidemia results from a previously colonizing *Candida* strain. Most evidence points to the importance of the patient's own gastrointestinal flora as the source of candidemia [33–40]. In hematological patients antibacterial treatment has been shown to promote intestinal colonization by fungi [33].

The exogenous origin of candidemia is implied when the causative pathogen is acquired from the hospital environment or a human source during a hospital

stay [41]. The transmission of exogenous *Candida* strains may occur through direct contact or indirectly via hospital personnel (e.g., hand-carriage of *Candida* strains of healthcare worker (HCW), thus inducing colonization and possibly infection [42–46]. The cutaneous (exogenous) origin of candidemia is particularly debated in association with *C. parapsilosis* candidemia and intravascular catheters, as well as in patients with extensive burns [13, 47]. The colonization of skin may also originate from the gastrointestinal tract, as has occurred in neonates [48]. The direct exogenous acquisition of candidemia has also resulted from contaminated intravenous solutions and medical devices [29, 49].

2.1.3 Definitions and types of candida infections

Colonization is defined as the multiplication of micro-organism at body sites of a host unaccompanied by clinical symptoms or immune response. Infection implies the replication of the pathogen in question in the tissues of a host, causing systemic or localized subclinical or clinical adverse reactions [50].

Nosocomial BSI is defined as the culture of a recognized pathogen in the bloodstream of a patient who has been hospitalized for >48 h, thus an infection that is absent or incubating when the patient is admitted to the hospital, in contrast to community-acquired BSI [51, 52]. Although >48 h after admission is generally deemed indicative of nosocomial acquisition of the infection in question, each case must be assessed individually for evidence that links it to hospitalization. Nosocomial BSIs may occur as outbreaks when the incidence rates increase above the expected or the usually observed rate [53].

The spectrum of diseases associated with *Candida* species is wide, and while superficial candidiasis of the skin and mucocutaneus surfaces are clearly the most frequent, candida may also cause life-threatening invasive infections, candidemia, and systemic or disseminated candidiasis. Candidemia refers to a condition where *Candida* species are isolated from the blood culture. Candidemia often involves disease outside of the bloodstream and frequently associates with a sepsis syndrome [54]. In clinical situations, as a rule, one positive blood culture for *Candida* spp. is deemed significant. Disseminated candidiasis, on the other hand, refers to a condition where an invasion of *Candida* is shown by culture or histology results of normally sterile tissues with or without candidemia [55].

The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and National Institute of Allergy and Infectious Diseases Mycoses Study Group have created an international consensus defining invasive fungal infections for immunocompromised patients with cancer and hematopoietic stem cell transplants intended for use in the context of clinical or epidemiological research [56]. This consensus classifies candidemias by their level of certainty for the diagnoses (i.e., as "proven" or "probable") in association

with mycological evidence from clinical specimens and temporally related clinical symptoms of infection.

2.1.4 Public health impact

Candidemia represents a considerable burden in hospitalized patients contributing significantly to morbidity, mortality and increased cost and length of hospital stay.

Morbidity

Candida has emerged worldwide as an important cause of nosocomial BSIs. In the USA during the 1980s, candidemias increased, accounting for 7–10% of all nosocomial BSIs, yet during the 1990s and 2000s, *Candida* spp. ranked the fourth most common causative pathogen in nosocomial BSIs [1–3, 57–61]. In Europe, candidemias increased during the 1990s, accounting for 3–9% of all nosocomial BSIs and ranking the seventh to eight most common causative pathogen isolated [5, 62–65]. In intensive care patients during the 1990s–2000s, *Candida* spp. caused 11–12% of nosocomial BSIs in the USA and accounted for 6–9% in Europe [66–70]. Candidemias accounted for 7–9% of all BSIs among adult and pediatric cancer patients [71, 72]. In HIV-patients, *Candida* spp. caused 19% of all nosocomial BSIs in a study from Italy, but highly active antiretroviral therapy has decreased the incidence of nosocomial infections these patiens [73, 74].

Mortality

The mortality associated with candidemia is substantial in both children and adults, much higher than should be expected to result from the underlying disease alone. The reported all-cause mortality has ranged from 30–61% in candidemia patients, and its attributable mortality from 5–49% [1, 6, 8, 75–78].

Cost and length of hospital stay

Candidemia has an enormous impact on the utilization of medical resources and costs. The largest component of total costs is the prolonged length of stay (LOS) in hospital. The increase in total hospital charges were USD 33,604–45,602 (mean USD 39,000) for an adult candidemia patient, USD 65,058–119,474 (mean USD 92,266) for a pediatric patient, and USD 1,374–76,715 (mean USD 39,045) for a neonate with candidemia in the USA [8, 78, 79]. Another report from the USA calculated an increase in total hospitals charges in the range of USD 6,000–29,000 per candidemia patient in a mixed patient population and USD 28,000 for a neonate with candidemia [6, 80]. A recent report from the USA estimated that each one-day delay in empirical therapy for candidemia was associated with increased total hospital costs of USD 6,392 ± 3,000 during 2002–2004 [81]. A Spanish study reported an additional cost of almost EUR 16,000 per patient due to *Candida* infection in

intensive care patients during 1998–1999 [7]. The reported increase in average LOS in hospitalized patients with candidemia has varied between 3–22 days and 4–12 days in intensive care patients [6–8, 75, 79].

2.1.5 Surveillance

Surveillance provides information on the incidence rates and outcome of candidemias as well as patterns of antifungal susceptibility of the causative pathogens, thus enabling the detection of outbreaks and assessment of the impact of prevention strategies and treatment. Surveillance systems differ between countries, which complicates the comparison of data they produce. Important issues include whether surveillance is active or passive; nationwide, regional or based on selected institutions; and statutory or voluntary. Most existing surveillance systems can be categorized into population-based or sentinel surveillance programs that target either the overall rates and causative pathogens of nosocomial infections (including BSIs), only infections caused by *Candida* species, or special patient groups at risk (such as intensive care patients).

Population-based surveillance

Population-based surveillance studies were conducted in the USA by the Centers for Disease Control and Prevention (CDC) and by the Emerging Infections and the Epidemiology of Iowa Organisms (EIEIO) surveillance program during the 1990s. Reporting was laboratory based, voluntary, and limited to a certain geographical area [23, 82–84]. In Europe, several countries have their own ongoing laboratory based, voluntary ,or statutory reporting system of bloodstream pathogens, which provides data for population-based incidence rates [17, 64, 85–87]. Also, during 2002–2003, a prospective population-based surveillance of candidemia was conducted in the greater Barcelona area in Spain [88, 89].

Sentinel surveillance

In the USA, the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) is an ongoing project that includes 49 sentinel hospitals geographically dispersed throughout the USA and collects data on nosocomial bloodstream pathogens and their antimicrobial susceptibility patterns as well as clinical data [3, 57, 59, 90]. The European Confederation of Medical Mycology (ECMM) executed a similar project in 106 institutions in seven European countries during 1997–1998 [12, 91, 92].

Surveillance in intensive care unit (ICU) patients

In 1970, the CDC established in the USA the oldest ongoing surveillance system, National Nosocomial Infections Surveillance (NNIS). A network of sentinel acute

care hospitals began to report all nosocomial infections to the NNIS on a voluntary basis [21, 67, 93, 94]. In addition to hospital-wide surveillance, three standardized protocols were initiated in the NNIS in 1986: the ICU, the high-risk nursery, and the surgical patient component. In 2005, the NNIS merged with two other healthcare surveillance systems at the CDC, The Dialysis Surveillance Network and the National Surveillance of Healthcare Workers, to establish the National Healthcare Safety Network (NHSN) [58, 95]. In Europe, a network for data on nosocomial infection rates in ICU patients was founded in 1994 by the Hospitals in Europe Link for Infection Control through Surveillance (HELICS), continued as part of the Improving Patient Safety in Europe project (IPSE), and transferred to the European Centre for Disease Prevention and Control (ECDC) in 2008 [69, 96–98]. During the 1990s, two more limited surveillance programs for ICU patients were executed: the National Epidemiology of Mycoses Survey (NEMIS) in the USA and the European Prevalence of Infection in Intensive Care (EPIC) in Europe [40, 68, 99–102].

Surveillance for microbiological data

Two international programs on the occurrence and antimicrobial resistance patterns of yeast pathogens causing bloodstream or other infections (i.e., the SENTRY Antimicrobial Surveillance program and the ARTEMIS DISK Global Antifungal Surveillance Study) are ongoing. Participating centers are dispersed globally, whereas the isolated microbes are tested centrally, thus enabling the direct comparison of strains between different geographical areas [103–110].

2.1.6 Risk factors

The list of reported risk factors for candidemia is extensive. Colonization with *Candida* spp. is considered a near-absolute requirement of candidemia (direct inoculation excluded), and the risk for infection may be related to the density and the extension of yeast colonization over time [111–114]. After colonization the main factor predisposing to candidemia is immunosuppression by disease or treatment, as in patients with haematological or solid malignancies, HIV patients, premature neonates, patients with extensive burns, and patients with transplants [47, 74, 115–121]. Thus, the patient groups at risk for candidemia have either severe underlying conditions impairing their host defences or are in need of invasive and intensive diagnostic procedures and medical or surgical treatments, or both. These predisposing factors may also evolve with changes in transplantation practices (e.g., new hematopoietic transplantation modalities and the technical complexity of surgery in liver transplantation) [122, 123].

Several prospective or retrospective cohort or case-control studies have identified multiple risk factors for candidemia in addition to prior colonization in different patient groups (ICU patients, neonates and patients with haematological or solid malignancies). Major risk factors include the use of central venous catheters

(CVCs); prolonged neutropenia; receipt of total parenteral nutrition (TPN), multiple bacterial antibiotics, or chemotherapy; gastrointestinal perforations and extensive abdominal surgery; renal failure or hemodialysis; and mechanical ventilation [35, 40, 99, 113, 124–129]. Both neutropenia and chemotherapy-induced injury to the gut wall increase the risk for candidemia in patients with malignancies, while catheters, invasive procedures, and perforation or surgery of the gut wall are more important factors in the non-cancer settings.

Because recognized risk factors are extremely common in the critically ill, clinical prediction rules have been created to select high-risk patients for candidemia [130–132] and the combinations of variables used include prior colonization, CVC or TPN, prior antibiotics, severe sepsis, hemodialysis, and major surgery.

Although rare, a study reported a genetic propensity for invasive *Candida* infections in humans: a homozygous point mutation in CARD9, which results in decreased numbers of Th17 cells (helper T-cells producing interleukin-17) in one family of five generations [133].

2.2 Diagnosing and typing methods for candidemias

The diagnostic strategy is to combine clinical signs, individual patient-related risk factors, and available laboratory data suggestive of *Candida* infection. While the gold standard for diagnosis is the detection and culture of causative yeast in clinical samples, new non-culture methods for earlier diagnosis, such as antigen assays and rapid PCR-based methods, are currently or will be in the future in clinical use. The investigation of outbreaks relies on molecular methods able to analyze strain relatedness.

2.2.1 Primary diagnosis

Culture

The fungal culture is the gold standard for diagnosis, but lacks sensitivity as up to 50% of blood cultures can be negative or become positive late in the course of infection [134, 135]. The detection of *Candida* spp. in blood culture systems depends on several variables, such as the volume of blood, the conditions of the medium and the atmosphere, the concentrations of yeast within the bloodstream, and the species of *Candida* in question [136]. Advances in blood culturing techniques to improve sensitivity and to reduce the time needed to obtain a positive culture include the development of lysis centrifugation tubes and specialized broth media as well as, importantly, the implementation of continuous-monitoring automated blood

culture systems (Bactec, Becton Dickinson, USA and BacT/Alert, Organon Teknika Corp., USA) [134, 136, 137].

Immunological and biological methods

Non-culture or serological methods to detect candidemia and disseminated candidiasis are generally based on the detection of circulating antigens or fungal metabolites. The clinical usefulness of antibodies has been limited because of false-positive results in patients with colonization and because immunosuppressed patients mostly produce low or undetectable antibody levels [134, 137].

Among the numerous potential target antigens, the highly immunogenic components of cell-wall antigens mannan and β-D-glucan are the most widely used and commercially available [134, 138, 139]. Mannan is the main component of the cell wall of *Candida* spp. and is rapidly cleared from the bloodstream, thus requiring regular sampling. Circulating mannan antigens detected with immunoassay, performed in parallel with antimannan antibody tests, have yielded a high sensitivity of 80% and a specificity of 93% in the diagnosis of candidemia and disseminated candidiasis [138]. β-D-glucan is part of the outer wall of many pathogenic fungi and is also ubiquitous in the environment, which may generate false-positive results [137, 138]. Most medically important *Candida* spp. (except *C. krusei* and *C. glabrata*) produce a metabolite known as D-arabinitol, which can be measured in serum and urine [134, 140]. The clinical significance of D-arabinitol detection is limited in guiding the initiation of antifungal therapy [141].

Molecular methods

Molecular methods targeting fungus-specific nucleic acids have been applied directly to clinical materials or to the contents of blood culture bottles to circumvent the time needed for culture and to increase the sensitivity of detection [134]. The use of specific oligonucleotide probes enables identification of an organism to the species level and can theoretically detect mixed fungal infections accurately. The amount of target yeast DNA available in clinical samples is typically very low, thus techniques used for detection necessarily rely on amplification [142]. These techniques include polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA). Currently, molecular strategies complement conventional methods and are used only in specialty laboratories.

Target sequences in PCR-based methods may be either species-specific, *Candida*-genus specific, or universal panfungal sequences. The ribosomal RNA (rRNA) gene most often serves as the target sequence, with sensitivity ranging from 78% to 100% for *Candida* infections while specificities vary [137, 142]. Nested PCR increases the sensitivity and specificity of detection, but at the expence of amplifying the occurrence of false-positive results [142]. Real-time PCR allows for rapid detection of the production of amplicons [142]. NASBA is a specific and sensitive RNA amplification technique capable of detecting six various *Candida* species.

Compared to PCR, NASBA detects only living yeast cells and needs no thermal cycling instrument, but is more expensive [142].

2.2.2 Species identification

Most yeasts are identified on the basis of carbohydrate assimilation and fermentation tests along with their macroscopic and microscopic morphologic features on specialized media [134, 137]. Rapid and less laborious commercially available methods have been developed, perhaps the most convenient consisting of fully automated system that evaluates an optical signal generated by individual biochemical reactions contained within a variety of microbe identification cards (VITEK 2, bioMérieux, Inc. Hazelwood, MO) [143, 144]. The addition of chromogenic substrates to the agar medium allows the direct detection of spesific enzymatic activities characteristic of selected species of *Candida* by their color and colony characteristics [145].

Molecular methods based on PCR, as described for the primary diagnosis of candidemia, also allow for species identification. Fluorescent in-situ hybridization (FISH) may serve to detect yeasts without the need for a pure culture [134, 142]. Although FISH is probably less sensitive and requires more time than PCR-based methods, it may be more economical as only positive samples are examined. Novel peptide nucleic acid (PNA) FISH requires only 2.5 hours after a blood culture is designated positive and is able to differentiate *C. albicans* from non-albicans *Candida* spp. (sensitivity and specificity 100%) as well as from *C. dubliniensis* [137, 142].

2.2.3 Susceptibility testing

Antifungal choice is based primarily on *Candida* spp. identification, but antifungal susceptibility tests play an increasingly important role in selecting which agent to use. Susceptibility testing also allows for resistance surveillance and comparison of the *in vitro* activity of new and existing agents. Methods used for susceptibility testing of yeasts include disc diffusion, agar dilution, and broth dilution procedures. Numerous *in vitro* factors such as media, buffer, inoculum, incubation and end point criteria may significantly affect the results [146].

In the USA, the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee on Clinical Laboratory Standards, NCCLS) has developed standard methods based on broth dilution and disc diffusion to test the susceptibility of yeasts [147, 148]. Althoug quality control and minimal inhibitory concentration (MIC) breakpoint criteria have been established for most antifungals, there is a concern that these methods fail to distinguish amphotericin B-resistant strains. [137, 146–148]. In Europe, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has developed a standardized broth dilution method for the susceptibility testing of yeasts with MIC breakpoint criteria established for

fluconazole and voriconazole [149]. Despite of methodological differences, these methods generate rather similar and comparable results [150]. Interpretive categories differ, however, between CSLI and EUCAST, as CSLI categorizes these as susceptible, susceptible-dose dependent, and resistant, while EUCAST assigns only susceptible or resistant categories.

Because these standardized methods are time consuming and laborious, more convenient techniques have been developed for routine susceptibility testing. The Etest method (AB Biodisk) is widely used and yields accurate, reproducible, and quantitative MIC results, but the values tend to be slightly higher than with broth dilution methods [151, 152]. Several other methods are commercially available as well as fully automated system that determines yeast growth spectrophotometrically [146, 150, 153–155].

2.2.4 Epidemiological typing methods for candida infections

Molecular methods enable assessment of the interrelatedness of clinical isolates and thus facilitate the tracing of sources and routes of transmission of infection outbreaks. A vast array of molecular typing methods is available, based on either nonamplifying techniques or those using DNA amplification by PCR, a summary of which appears in Table 1. All systems require a pure culture of each isolate, yeast cell lysis, DNA extraction, and the use of electrophoresis to separate DNA fragments into patterns (fingerprints), which are then visualized by staining or by hybridizing with labelled probes [156].

Most fungal species display chromosome-length polymorphisms, which can be analyzed by electrophoretic karyotyping (EK) [142, 156]. The development of pulsed-field gel electrophoresis (PFGE) allows the separation of even extremely large DNA fragments and allows for high reproducibility and discriminatory power, thus making it currently the method of choice in most outbreak investigations [156]. In restriction enzyme analysis (REA) and in restriction fragment length polymorphism (RFLP), DNA is cleaved by selected endonucleases into small fragments, which are then separated with electrophoresis. RFLP is rapid, easy, and inexpensive, but restriction patterns are very complex and difficult to compare [142]. For easier comparison, separated fragments can be transferred onto a membrane and hybridized with a labeled probe [142, 156].

Random amplified polymorphic DNA (RAPD), also referred to as arbitrarily primed PCR (AP-PCR), uses arbitrarily chosen primers to amplify nearly homologous sequences of genomic DNA. Strains are then differentiated by determining the characteristic number and size of the amplified fragments with electrophoresis [142, 156]. While RAPD is easy, technically simple and fast, minor differences in experimental conditions can result in different profiles, thus reducing reproducibility. Amplified-fragment length polymorphism (AFLP) based on the

selective amplification of a subset of DNA fragments generated by restriction endonucleases, then separated by gel electrophoresis and analyzed [156]. AFLP is not only a highly reproducible and robust method, but is also easier to read than RFLP. Multilocus sequence typing (MLST) is based on the nucleotide polymorphism of internal fragments of six to eight independent chromosomal genes [157]. MLST schemes have been developed for five *Candida* species, and maintained as an open platform.

TABLE 1. Comparison of performance characteristics of epidemiological typing methods for *Candida* species (modified from [142, 156]).

	Advantages	Disadvantages	Time required (days)	Comments
Non-amplified				
EK[a] with PFGE[b]	high reproducibility high discriminatory power	laborious expensive equipment	3–5	widely used
REA[c], RFLP[d] (conventional electrophoresis or PFGE[b])	rapid easy inexpensive	complex banding patterns laborious	2	
Amplified				
RAPD[e] or AP-PCR[f]	high discriminatory power universally applicable rapid easy inexpensive	lack of reproducibility	1	widely used
AFLP[g]	reproducible high discriminative power automation	laborious expensive needs expertise	2	
MLST[h]	high discriminative power reproducible rapid	technically demanding expensive applicable to 5 Candida species	2	

[a]EK = electrophoretic karyotyping; [b]PFGE = pulsed-field gel eletrophoresis; [c]REA = restriction enzyme analysis; [d]RFLP = restriction fragment length polymorphism; [e]RAPD = random amplified polymorphic DNA; [f]AP-PCR = arbitralily primed polymerase chain reaction; [g]AFLP = amplified fragment-length polymorphism; [h]MLST = multilocus sequence typing.

2.3 Epidemiology of candidemia

2.3.1 Incidence

The incidence of candidemia has mostly been studied in population-based or in-hospital settings. Although population-based data are representative of the whole population living in the area studied and contribute to a more comprehensive data set, inclusion of both inpatients and outpatients, and the lack of selection bias that is inherent in tertiary care hospital-based studies, they are expensive and laborious, and so are essentially limited to a specific geographical area and time period. Sentinel surveillance programs are more flexible, however, and allow sampling of consecutive episodes of infection from a large number of institutions over a broad geographical area, and for a prolonged period of time [158].

Population-based studies

A summary of population-based studies appears in Table 2. The first population-based studies reported high average annual incidences between 8.0–7.3 per 100,000 population in selected urban areas in the USA during 1992–1993 [83, 84]. Subsequent studies observed great variation in incidences between urban and rural areas (24 vs. 7.0–6.0 per 100,000 population) [23, 82]. Also, incidence rates in Canada differed across separate areas and increased over time in Calgary [11, 159]. In Australia, the annual incidence rate was low (1.8 per 100,000 population) with wide variation between different parts of the continent [160].

In Iceland, during 26 years of observation, the annual incidence was low but rose; in Norway, during 13 years of observation, annual incidence remained low and constant at 2.4 per 100,000 population [17, 86, 161]. In Denmark, however, incidence rates have been much higher: 11 per 100,000 population, during the 2000s [85, 162]. Elsewhere in Europe, reported annual incidence rates have been somewhat higher than in Scandinavia, but lower than in the USA [87, 89]. In the UK, the incidence of candidemia increased during 2004–2007, but considerable differences have been noted between different areas, with rates in Northern Ireland being much higher than in Wales or England [87].

The highest age-specific incidence rates have been observed in infants <1 year of age 75 per 100,000 population in the USA and in Canada, and somewhat lower in Europe, 10.3–38.8 per 100,000 population [11, 17, 83, 85, 86, 89]. The incidence is low in patients aged ≥1–40 years but subsequently increases with advancing age hence the annual incidence in the range of 7.4–36.9 per 100,000 population in patients >65 years of age [17, 83, 85, 86, 89]. Male dominance in all age groups is common globally, though not constantly [11, 23].

TABLE 2. Summary of population-based studies reporting annual incidences of candidemias and the proportion of non-*C. albicans* species.

Country/area	Time period	Catchment population	Incidence per 100,000 population	Proportion of non-*C. albicans* spp.	Reference	Remarks
USA/Atlanta, San Francisco	1992–1993	5.34 million	8.0	44–51%	[83]	
USA/California	1992–1993	2.94 million	7.3	not available	[84]	
USA/	1998–2000	4.7 million	10.0	55%	[23]	
Baltimore			24.0			
Connecticut			7.0			
USA/Iowa	1998–2001	not available	6.0 (5.6–6.8*)	not available	[82]	
Canada/	1992–1994	2.22 million	3.3	31%	[159]	
Hamilton			5.1			
Ottawa			1.2			
Manitoba			3.5			
Canada/Calgary	1999–2004	~1 million	2.8	49%	[11]	
Australia	2001–2004	not available	1.8	53%	[160]	2 of 52 laboratories missing
Norway	1991–2003	~4.3million	2.4 (2.0–3.3*)	30%	[17]	
Iceland	1980–1999	0.285 million	1.4–4.9*	32%	[86]	
Iceland	1991–2006	~0.281 million	3.3–5.8*	38%	[161]	
Denmark	2003	2.8 million	11.0	35%	[162]	
Denmark	2004–2006	3.5 million	10.4	38%	[85]	
Spain/Barcelona	2002–2003	3.9 million	4.3	49%	[89]	
UK	2004–2008	not available	3.2–3.6*	35–36%	[87]	voluntary reporting
England			3.1–3.5*			
Wales			2.7–3.1*			
Northern Ireland			5.0–6.8*			

*Range per year.

In-hospital studies

As Table 3 shows, candidemia rates vary according to the characteristics of the patient population considered and the type of institution. The comparison of rates is complicated by differences in calculated rates (i.e., per patient days or per admissions or discharges). Incidence rates are lower in general hospitals than in tertiary care centers (TCCs), and are probably best shown as rates per 1,000 patient days (incidence densities).

In the USA, the incidence rates for candidemia and disseminated candidiasis rose considerably during the 1980–1990s both in general hospitals and in TCCs, and have remained stable, 0.02 per 1,000 admissions, during the 2000s [1, 2, 163–165]. In Europe, average annual incidence rates have varied between 0.02–0.07 per 1,000 patient days or 0.2–0.5 per 1,000 discharges in general hospitals during the 1990–2000s [12, 89, 92, 166–169]. In 5 TCCs in the Netherlands during 1987–1995, annual incidence rose over time from 0.03 to 0.07 per 1,000 patient days, while in 17 TCCs in Switzerland the annual incidence remained stable 0.05 per 1,000 patient days during 1991–2000 [5, 65]. In Europe, many of the reports are from single centers over a short period of time and while some of the studies covering a longer period of time have observed the increase in incidence [170], others have not [14, 75, 171]. Only one center reported a decrease in incidence from 0.2 to 0.1 per 1,000 patient days during 2001–2005 [172]. In Australia (Queensland), the average annual incidence rose 7.6-fold (from 0.007 to 0.05 per 1,000 patient days) in general hospitals and 2.9-fold (from 0.03 to 0.1 per 1,000 patient days) in adult TCCs during 1999–2008 [173]. At 0.4 per 1000 patient days, the observed average incidence of candidemia has been higher in TCCs in Brazil than both in the USA or Europe [174, 175]. On the other hand, in Taiwan, in one TCC, the incidence of candidemia has been rising constantly during 1981–2000 [176].

The incidence of candidemia is higher in ICU patients than in general wards, but rates vary considerably between institutions and according to predominant type of patients receiving care in the ICU (i.e., neonatal, pediatric, and adult medical or surgical ICU). In neonatal ICUs (NICUs) the observed rates have been high, 12.3 per 1,000 admission or 0.6 per 1000 patient days in the USA [40, 100, 118]. In Europe, the incidence rates among NICU patients have been lower, 11 per 1,000 admissions or 0.03 per 1,000 patient days [88, 172]. The reported incidence rates in pediatric ICUs (PICUs) have been in the same range (0.02 per 1,000 patient days or 0.6 per 1000 CVC days) [5, 15]. Reported incidence rates in adult ICU patients have been high, 1 per 1,000 patient days or 9.8 per 1000 admissions in the USA, but during 1989-1999 a decreasing rate was observed, varying between the 0.7–0.5 per 1000 CVC days [15, 100]. In Europe the rates among adult ICU patients have varied greatly from 2.0 to 6.7 per 1,000 admissions or from 0.03 to 0.2 per 1,000 patient days [5, 14, 172, 177–179]. In Australia and Taiwan, the reported incidences among adult ICU patients have been similar to Europe [5, 14, 172, 173, 177–180]. Reported annual incidences for candidemia in cancer centers have varied from 5.2 to 6.0 per 1,000 admissions [13, 181]. In hematology units reported incidences have varied from 0.05 to 0.9 per 1,000 patient days [9, 168, 172, 178].

TABLE 3. Summary of in-hospital studies reporting incidence rates and the proportion of non-*C. albicans* species.

Country/Type of hospital	Number of hospitals	Time period	Incidence rate/		Proportion of non-*C. albicans* spp.	Case fatality[b]	Reference	Remarks
			1,000 patient days	1,000 admissions[a]				
General hospitals								
USA/NHDS[c]		1980–1989		0.01–0.15[c]	NA[e]	45%	[164]	disseminated candidiasisf
USA/NNIS[g]		1980–1989		0.3	NA	NA	[163]	
USA/NHDS[c]		1996–2003		0.2	NA	NA	[2]	disseminated candidiasisf
Norway		1991–1996	0.03	0.2	34%	NA	[169]	~96% candidemias
Israel	14	1994		0,4	46%	22%	[167]	all candidemias
France	25	1995	0.04	0.3	47%	NA	168]	nosocomial ~70%
Europe/ECCM[h]	106	1997–1999			44%	38%	[12]	all candidemias
France	18			0.2 (0.04–1.3[i])	NA			
Germany/ Austria	5		0.03 (0.01–0.05[i])		NA			
Sweden	6			0.3 (0.1–1.4[i])	33%	31%	[91]	
Italy	32	1997–1999	0.04	0.4	42%	35%	[92]	nosocomial 97%
France		1998–2001		0.3	50%	NA	[166]	all candidemias
Spain	14	2002–2003	0.07	0.5	49%	44%	[89]	all candidemias
Australia	167	1999–2008	0.05 (0.02–0.07[d])	0.15	55%	NA	[173]	all candidemias
Australia	40	2001–2004		0.2	53%	NA	[160]	nosocomial 82%

Country/Type of hospital	Number of hospitals	Time period	Incidence rate/ 1,000 patient days	Incidence rate/ 1,000 admissions[a]	Proportion of non-*C. albicans* spp.	Case fatality[b]	Reference	Remarks
Tertiary care centers								
USA/NNIS[g]		1980–1989		0.4–0.6[i]	NA	NA	[163]	
USA	1	1983–1986		0.9	39%	57%	[1]	
USA	1	1987–1994	0.04–0.10[d]		49–23%	NA	[165]	
Netherlands	5	1987–1995	0.03 (0.03–0.07[d])		33%	NA	[65]	all candidemias
Spain	1	1988–2000		0.6	49%	NA	[14]	nosocomial
Switzerland	17	1991–2000	0.05		34%	NA	[5]	
Italy	1	1992–1997	0.1 (0.10–0.12[d])		46%	45%	[16]	
Belgium	1	1994–2004	0.09 (0.06–0.14[d])		37%	NA	[182]	
UK	2	1995–2001		0.3 (0.2–0.4[d])	36%	35%	[170]	nosocomial
France	9	1995	0.05	0.4	49%	NA	[168]	nosocomial ~70%
Spain	1	1995–1997		0.76	54%	44%	[183]	all candidemias
Spain	1	1995–1999		0.81	56%	45%	[171]	all candidemias
Ireland	1	1999–2003	0.07 (0.06–0.08[d])	0.5 (0.4–0.6[d])	50%	40%	[184]	all candidemias
Belgium	1	2001–2005	0.2 (0.2–0.1[d])		41%	NA	[172]	nosocomial fungemia
Portugal	1	2004		2.0	NA	39%	[185]	all fungemias
Taiwan	1	1981–2000		0.08–0.3[d]	40–60%	NA	[176]	nosocomial
Canada	3	1992–1996		0.05 (0.2–0.8[i])	46%	46%	[186]	all candidemias
Australia	1	1992–1999	0.05	0.27	47%	45%	[9]	nosocomial
Australia	8	1998–2008	0.6 (0.03–0.09[i])		55%	NA	[173]	all candidemias
Brazil	4	2002–2003		1.7 (1.5–2.0[i])	62%	61%	[174]	nosocomial 89%
Brazil	11	2003–2004	0.4 (0.2–0.5[i])	2.5 (1.5–5.3[i])	59%	54%	[175]	all candidemias

[a]admissions or discharges; [b] within 30 days, during hospitalization or not defined; [c]NHDS = National Hospital Discharge Survey; [d]range by year; [e]NA = not available; [f]discharge data; [g]NNIS = National Nosocomial Infections Surveillance; [h]ECMM = European Confederation of Medical Mycology; [i]range by hospital.

2.3.2 Causative species

The most abundant causative species, by far, is *C. albicans* (Tables 2 and 3), but many centers reported a worldwide shift during the 1990s towards non-*C. albicans* species [13–15, 23, 65, 83, 85]. Both population-based and in-hospital studies have reported this shift in causative species, yet many studies have observed no change in causative species over time [5, 17, 182]. International surveillance studies have observed marked geographical variation in the distribution of causative species of candidemia, with *C. glabrata* ranking second and *C. parapsilosis* third in the USA; in Canada and Europe *C. parapsilosis* ranked second before *C. glabrata*, and in Latin America *C. glabrata* was rarely isolated [22, 103, 104]. The species distribution also differs by demographics and the type of the patient population observed, as *C. parapsilosis* is most abundant in neonates, *C. glabrata* is observed mostly in adults and older age groups and *C. krusei* in cancer patients [12, 13, 82, 118, 160].

2.3.3 Outbreaks in specific hosts

Outbreaks of candidemia stemming from a single strain are uncommon, but several reports have described nosocomial clusters in different patient populations. Horizontal transmission from patient to patient or acquisition from the hospital environment in ICUs and the bone marrow transplant unit has been documented for *C. albicans*, *C. glabrata*, and *C. parapsilosis* strains [44–46, 187–189]. The principal mechanism of transmission is most probably indirect contact via HCWs hands, but other mechanisms include the contamination of intravenous fluids and medical devices [49, 190–192].

Outbreaks in neonates

C. parapsilosis has caused several nosocomial clusters of candidemia in neonates. The longest observation period has been 12 years, with the domination of a single strain of *C. parapsilosis* in one NICU, while most studies report several strains (genotypes) responsible for a short outbreak [193–196]. In one study, the source of transmission was liquid glycerine suppositories, and another identified the mecanism as the hand carriage of causative strains but the sources have largely remained elusive [193, 196]. *C. albicans* has also caused outbreaks of candidemia in neonates, and one study reported hand carriage of the causative strain [197–199]. Two studies have attributed outbreaks in neonates to several *Candida* spp. [200, 201]. Another study associated an outbreak with the use of topical petrolatum ointment, while yet another study identified retrograde medication syringes contaminated by the causative strain.

Outbreaks in critically ill and surgical patients

Candidemia outbreaks in critically ill patients have mostly been caused by *C. parapsilosis* [202–205]. The cause of these outbreaks has probably been multifactorial, as seriously ill patients often require invasive ventilation and monitoring, and thus frequent contact with HCWs, which facilitate the cross-transmission of causative yeasts and magnifies the impact of lapses in hand hygiene [202]. One study identified hyperalimentation infusion fluid as a common source of infection [205]. *C. albicans* outbreaks in critically ill patients have been associated with hand carriage of the causative strain [206, 207]. One report documents an outbreak caused by a single strain of *C. rugosa* in a burn unit [208]. *C. parapsilosis* has been implicated in two candidemia outbreaks consisting of five and four patients, respectively, in patients with prosthetic valve surgery [209, 210]. In the first outbreak in 1984, *C. parapsilosis* was cultured from cardiac bypass equipment, while the second outbreak probably stemmed from frequent glove tears during surgery, thus facilitating transmission of the yeast to patients. One report revealed the persistence of a single *C. albicans* strain for four years in a surgical ward causing eight candidemias [211]. Short BSI outbreaks caused by *C. albicans* in surgical patients have been associated with intravenous fluids or anesthetic agents [212, 213].

Outbreaks in patients with hematological or solid malignancies

Several outbreaks among haematological or cancer patients have been attributed to *C. parapsilosis* and associated with hand carriage of the causative strain in HCWs [41, 214–216]. One study reported an extensive *C. krusei* outbreak in the Netherlands consisting of 13 patients with candidemia and another 26 patients with colonization [217]. The outbreak was polyclonal, but the same genotype of *C. krusei* was identified in 25 out of the 39 patients, while all environmental cultures were negative. A cluster of 12 invasive C. krusei infections was detected within 9 months in a hematological unit, but nosocomial spread could not be shown [218].

2.3.4 Outcome

The case fatality ratio or rate (i.e., all-cause or crude mortality) is the ratio of the number of deaths from candidemia divided by the number of candidemia cases, expressed as a percentage [50]. Mortality rate is the number of deaths in candidemia patients divided by the number of individuals in a defined population during a certain period of time, such as a year (annual mortality rate), but the term is still often used to replace case fatality in the published literature. Reported case fatality ratios differ in terms of the observation period they use, thus complicating comparisons. Most report in-hospital crude mortality, but case fatalities over one week (7 days) and one month (28 to 30 days) are also common in the published literature. Attributable mortality refers to deaths directly related to candidemia in contrast to all-cause mortality.

Studies have reported high all-cause mortality among candidemia patients, ranging from 22% to 45% in a series of general hospitals and from 31% to 61% in a series of TCCs (Table 3). The case fatality ratios of candidemia have remained high during the past three decades despite new antifungals: only one study reported a significant decrease over time from 59% during 1980–1984 to 27% (per 30 days) during 1995–1999 in Iceland [219]. In matched cohort and case-control studies, the in-hospital mortality rate attributable to candidemia has varied between 15–49% in hospitalized patients in general, 5–31% in ICU patients, and 10–12% in pediatric patients and neonates [1, 6, 8, 75, 76, 78, 220]. The considerable variation in figures may exist because, although mortality associated with candidemia is high, this condition usually affects patients with severe underlying disease and thus a grim prognosis.

The risk factors for death among patients with candidemia include age ≥70 years, treatment for hematological or solid tumor, and candidemia caused by *C. glabrata, C. krusei,* or *C. albicans* [12, 16, 91, 92, 121, 167, 171, 183]. Among hematological and cancer patients, additional factors include prolonged neutropenia, allogeneic bone marrow transplantation, septic shock, and lack of antifungal prophylaxis, while high APACHE II scores in critically ill contribute to high crude mortality [10, 121, 181, 183, 221]. Regarding treatment, failure to promptly remove CVC and delay in the initiation of adequate antifungal treatment have increased crude mortality in candidemia patients [75, 115, 222–224].

2.4 Prevention and treatment strategies

2.4.1 Preventive measures and prophylaxis

Given the substantial excess mortality due to candidemia and the difficulties encountered in the early diagnosis and administration of effective antifungal treatment, the focus has been on prevention [24, 225]. Three low-tech strategies should be the primary approach to reducing the burden of nosocomial candidemia: improved hand hygiene, optimal catheter care, and prudent antimicrobial use [2, 24, 226]. Reducing the duration of neutropenia by using hematopoetic growth factors and curtailing mucosal damage resulting from herpes simplex infections are suggested options, but both require further study [226]. Considering that candidemia arises from endogenous sources, a rational preventive measure would be to reduce colonization with chemoprophylaxis. In ICU patients, a nosocomial setup with a high level of activity point to difficulties in implementing infection control practices: thus decreasing hand contamination of HCWs and strict hygienic practices in using invasive monitoring would be best methods. Alcohol-based hand rubs decrease hand contamination more effectively than use of soap and water [227, 228].

Antifungal prophylaxis should always be considered secondary to the standard infection control strategies and should be applied only when the rate of candidemia

remains elevated despite the assiduous application of these measures [24]. Antifungal prophylaxis has proved effective in decreasing colonization and invasive candidiasis, and at least a trend towards a reduction in associated mortality among neutropenic patients has been observed, while the benefits and cost-effectiveness of this strategy in nonneutropenic patients treated in ICU remain incompletely defined [2, 24, 225, 229–239]. Also, the issue of selecting for fluconazole- or triazole-resistant strains of *C. glabrata* and *C. krusei* should be addressed locally when choosing antifungals for use in prophylaxis.

The Infectious Diseases Society of America (IDSA) has published guidelines for using antifungal prophylaxis in different patient populations, including solid-organ recipients, hematological patients, the critically ill, and neonates (Table 4), along with the grading of evidence (Table 5) [229]. The Immunocompromised Host Society (ICHS), together with the European Organisation for Research and Treatment of Cancer (EORTC), the European Group for Blood and Marrow Transplantation (EBMT), and the European Leukemia Net have all contributed to the development of the European guidelines (the 3rd European Conference on Infections in Leukemia, ECIL) for prophylaxis and the management of bacterial and fungal infections in hematological patients (Table 4) [240]. Both of these guidelines regard antifungal prophylaxis in the primary prevention of invasive yeast and mould infections as the standard of care in acute leukemia patients and allogeneic stem cell transplant recipients during the neutropenic phase. Antifungal prophylaxis is recommended postoperatively for high-risk liver, pancreas, and small-bowel transplant recipients [229]. For critically ill adult patients, prophylaxis is recommended only to high-risk patients (e.g., patients with gastrointestinal surgery, CVCs, broad-spectrum antimicrobial agents, and multiple sites of candida colonization) in units with a high incidence of invasive candidiasis [229, 241]. In nurseries at high risk for invasive candidiasis, fluconazole prophylaxis is recommended to neonates with birth weights <1,000g.

2.4.2 Preemptive and empiric treatment strategies

Lack of specific clinical findings and slow, insensitive diagnostic methods complicate the early recognition and treatment of candidemias, while fungal BSIs have been shown to have some of the highest rates of inappropriate initial therapy and hospital mortality among all examined etiological agents of BSI [2, 224]. These factors have prompted the development of empirical and preemptive strategies for the treatment of candidemia. Different definitions exist, but most commonly preemptive therapy refers to the early treatment of infection with the use of clinical, laboratory, or radiological surrogate markers of disease in a high-risk host before clinical signs and symptoms of overt disease develop. Empirical therapy refers to the treatment of high-risk hosts who exhibit signs and symptoms of the disease, even in the absence of positive cultures or other evidence of disease [2, 242].

Empirical antifungal therapy is the standard of care among neutropenic cancer patients with persistent fever despite the administration of broad-spectrum antimicrobial agents [229, 240]. Preemptive antifungal therapy triggered by a radiological or serum marker has been proposed as a cost-effective substitute for empirical antifungal therapy in febrile, neutropenic patients [243]. Early initiation of antifungal therapy may reduce morbidity, mortality, and length of stay in critically ill patients, but the widespread use of these agents must be balanced against the risk of toxicity, costs, and the emergence of resistance. Empirical antifungal therapy should also be considered in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever [229, 241, 244, 245].

CVCs should be removed when candidemia is documented, if logistically feasible. The data supporting removal of CVCs are strongest among non-neutropenic patients and show that catheter removal is associated with shorter duration of candidemia and reduced mortality in adults and neonates [229]. The data on catheter removal in neutropenic patients with candidemia are less compelling [246, 247].

TABLE 4. Summary of published guidelines for the use of antifungal prophylaxis in different patient populations [229, 240]

Patient group at risk	Recommendation	Duration
	IDSA*	
Patients with chemotherapy induced neutropenia	fluconazole 400mg (6mg/kg) daily (AI) posaconazole 200mg 3 times per day (AI) caspofungin 50mg daily (BII) oral itraconazole 200mg daily (AI)	During induction chemotherapy for the duration of neutropenia
Allogeneic stem cell transplant recipients	fluconazole 400mg (6mg/kg) daily (AI) posaconazole 200mg 3 times daily (AI) micafungin 50mg daily (AI)	During the period of risk of neutropenia
Solid-organ transplant recipients Liver (high-risk) (AI) Pancreas (BII) Small bowel (BIII)	fluconazole 200-400mg (3–6mg/kg) daily liposomal amphotericin B 1–2mg/kg daily	≥7–14 days postoperatively
ICU**patients Adult high-risk patients in units with high incidence of invasive candidiasis (BI) Neonates with birth weight <1000g	fluconazole 400mg (6mg/kg) daily fluconazole 3–6mg/kg twice weekly (AI)	

Table 4 continues

Patient group at risk	Recommendation	Duration
	ECIL* **	
Patients with induction chemotherapy of acute leukemia	fluconazole 50–400mg daily iv/oral (CI) itraconazole oral solution 2.5mg/kg twice daily (CI) posaconazole 200mg 3 times daily (AI) polyene iv (CI) aerosolized amphotericin B+ oral fluconazole (BI) candins iv: insufficient data	
Allogeneic stem cell transplant recipients	fluconazole 400mg daily iv/oral itraconazole 200mg iv followed by oral solution 200mg twice daily (BI) micafungin 50mg daily (CI) polyene iv (CI) voriconazole 200mg twice daily (provisional AI) aerosolized lisomal amphotericin B+fluconazole (BII) posaconazole oral: no data	During the neutropenic phase
Allogeneic stem cell transplant recipients	fluconazole 400mg daily iv/oral (CI) itraconazole 200mg iv followed by oral solution 200mg twice daily (BI) posaconazole 200mg 3 times daily (AI) polyene iv (CI) voriconazole 200mg twice daily oral (provisional AI) aerosolized liposomal amphotericin B+fluconazole and candins iv: insufficient data	During graft-versus host disease phase

*IDSA = Infectious Diseases Society of America; ICU = intensive care unit; ***ECIL = European Conference on Infections in Leukemia.

TABLE 5. The grading system for ranking recommendations in clinical guidelines [229, 240].

Category; grade	Definition
Strength of recommendation	
A	Strong evidence for efficacy and substantial clinical benefit. Strongly recommended
B	Strong/moderate evidence for efficacy, but only limited clinical benefit. Generally recommended
C	Insufficient evidence for efficacy; optional
Quality of evidence	
I	Evidence ≥1 properly randomized, controlled trial
II	Evidence from ≥1 well-designed clinical trial without randomization; or cohort of case-controlled analytic studies (preferably from >1 center); or from multiple time-series; or from dramatic results from uncontrolled experiments
III	Evidence from the opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

2.5 Candidemia in Finland in light of previous studies

A summary of previous Finnish studies of candidemia appears in Table 6. Candidemias accounted for 4% of nosocomial BSIs in a surveillance study of hospitalized patients, although the proportion was higher (6%) in pediatric patients [64, 248]. During 1980s yeasts (95% *Candida* spp.) were detected in 0.2% of all blood samples taken for culture, and during 1989-1991, candidemia was observed in 1.9% of adult patients who underwent abdominal surgery [249, 250]. Candidemia has also been reported in 1.3% and 0.3% of adult allogeneic or autologous stem cell transplant recipients, respectively, while *Candida* spp. accounted for 0.4% of episodes of neutropenic fever among adults treated for acute myeloid leukemia [251–253]. Fluconazole prophylaxis was instituted in only 10% of autologous stem cell recipients. As for children, candidemia occurred in 3.4% of stem cell transplant recipients, 70% of whom received systemic antifungal prophylaxis [254]. Two studies which specially evaluated antifungal prophylaxis and which were executed in hematological patients, both found a low rate of candidemia and no evidence of a trend towards non-*C. albicans* spp [235, 255].

Reports from Finland have identified two nosocomial clusters of candidemia caused by *Candida* spp. A *C. parapsilosis* outbreak in one NICU, caused by a single strain with decreasing susceptibility to fluconazole, has persisted since 1987 [194,

256]. A cluster of invasive *C. krusei* infections occurred in twelve patients, among whom six candidemias were investigated in a hematological ward [218]. This cluster was caused by several *C. krusei* strains: the nosocomial spread could not be shown.

Hepatosplenic candidiasis was studied extensively among adult leukemia patients during 1980–1993 in Finland, while candidemia was diagnosed in only six patients [257].

TABLE 6. Summary of Finnish studies on candidemias in different patient populations

Study population	Number of infections/ patients/blood cultures	Hospitals	Years	Proportion of candidemias	Reference
Nosocomial BSI[a] in Finnish Hospitals	1,477	Selected hospitals/ SIRO[b]	1999–2000	4%	[64]
Nosocomial BSI[a] in children	739	HCA[c]	1999–2006	6%	[248]
Adult febrile patients <24h after abdominal surgery	107	Turku University Hospital	1989–1990	1.9%	[250]
Adult allogeneic transplant recipients	685	HUCH[d]	1983–2002	1.3%	[252]
Adult autologous stem cell recipients	1,188	Six Finnish transplant centers (nation-wide)	1990–2001	0.3%	[253]
Episodes of neutropenic fever in adult AML[e] patients	280	Kuopio University Hospital	1996–2005	0.4%	[251]
Pediatric stem cell transplant recipients	148	HCA[c]	1986–1996	3.4%	[254]
Pediatric hematology patients[f]	98	HCA[c]	2000–2002	1%	[255]
Adult acute leukemia patients[g]	1,089	HUCH[d]	1978–2004	8.7% vs 1.6%	[235]
Blood cultures	35,618	Turku University Hospital	1981–1986	0.2%	[249]

[a]BSI = blood stream infection; [b]SIRO = Finnish Hospital Infection Program; [c]HCA = Hospital for Childrean and Adolescents; [d]HUCH = Helsinki University Central Hospital; [e]AML = Acute myeloid leukemia; [f]prophylaxis with itraconazole oral solution 5mg/kg daily or fluconazole 5–8mg/kg daily oral/iv; [g]prophylaxis with fluconazole 400mg daily since the year 2000.

3 Aims of the study

The purpose of this study was to provide comprehensive data on the population-based incidence and outcome of *Candid*a bloodstream infections in Finland. With special emphasis on the largest tertiary care centre in Finland, the Helsinki University Central Hospital (HUCH), we assessed the impact of these infections in different patient groups at risk.

The specific objectives were:

1. To evaluate trends in BSIs caused by *Candida* in Finland during two periods 1995–1999 and 2004–2007 in a population-based (nation-wide) study, and to further analyze the characteristics of candidemia cases according to causative species and outcome. (I, II)
2. To evaluate trends and regional differences in fluconazole consumption and to describe prophylaxis policies in tertiary care regions in Finland during 2004–2007. (II)
3. To evaluate incidence rates, causative species, outcome and different patient populations at risk for nosocomial candidemia, as well as fluconazole consumption at the Helsinki University Central Hospital during 1987–2004. (I, III)
4. To investigate the epidemiological value of REA in *C. albicans* BSIs at the Helsinki University Central Hospital during 1986–1995. (IV)

4 Materials and methods

A short summary of the study methods, time periods and settings of studies I–IV appears in Table 7.

TABLE 7. Overview of the data sources, time periods and patients of the studies.

Study	Data sources	Time period	Setting	Patients or episodes/ isolates
I	NIDR*	1995–1999	Nationwide	479 candidemia episodes
	Laboratory-based identification of candidemia Review of patient charts	1995–1999	HUCH#	86 patients characteristics and outcome
II	NIDR* in connection with the National Population Information System	2004–2007	Nationwide	603 candidemia episodes and outcome
	National Agency of Medicine	2000–2007	Nationwide	Use of fluconazole
	Telephone survey	2004–2007	Five tertiary care hospitals	Prophylaxis policies
III	Laboratory-based identification of candidemia Review of patient charts	1987–1998	HUCH#	358 patients characteristics and outcome
	SIRO**	1999–2004	HUCH#	140 candidemia episodes and outcome
	Pharmacy	1991–1999 2003–2004	HUCH#	Fluconazole consumption in grams
IV	Laboratory-based identification of *C. albicans* bloodstream isolates	1986–1995	Helsinki-Uusimaa Health District	142 *C. albicans* isolates in 130 patients

*NIDR = National Infectious Diseases Register; **SIRO = Finnish Hospital Infection Program; #HUCH = Helsinki University Central Hospital.

4.1 Surveillance methodology for bloodstream infections of the National Infectious Disease Register (NIDR) (I–II)

In Finland (population 5.3 million), the national health care system is organized into five geographically and administratively defined tertiary care districts, each with a population ranging from 0.72–1.76 million. Since 1995, all clinical microbiology laboratories are required to notify all bacterial and fungal isolates from blood, including *Candida* species, to the National Infectious Diseases Register (NIDR), currently mostly electronically. Data collected with each notification include the date of specimen, isolated microbe, date of birth, sex, place of treatment, and (since 2004) each individual's unique national identity code. A case of candidemia was defined as a patient with at least one blood culture that tested positive for *Candida* species. Notifications of the same *Candida* spp. within three months of the first diagnostic sample in the same person were defined as one case. Isolation of the same species beyond this time period was defined as separate cases. Through each patient's unique national identity number the data collected during 2004–2007 were linked to the National Population Information System to obtain a particular patient's survival at 7 and 28 days from the first positive blood culture (Study II).

4.2 Description of the hospitals, charts review, and case definitions (I, III, IV)

Helsinki University Central Hospital (HUCH) is a tertiary care center with ~1,700 beds serving a population of 1.66 million in Helsinki and surrounding areas in Southern Finland. In some specialties, such as stem cell and solid organ transplantations, HUCH provides national services. During the study period 1987–2004 (III), solid organ and allogeneic stem cell transplantation more than doubled in HUCH, from 161 per year in 1987 to 372 per year in 2004. Autologous stem cell transplantations increased from 11 per year in 1987 to 95 per year in 1997, and thereafter until 2004, varied between 89 and 50 per year (III).

All patients with at least one blood culture that tested positive for *Candida* spp. during years 1986–1999 (I, III) and with stored blood culture isolates of *C. albicans* between November 1986 and May 1995 (IV) were retrospectively identified from the microbiology laboratory logbooks. The following data were retrieved from patient charts: date of isolation, place of treatment (HUCH and other acute care hospitals in the Helsinki-Uusimaa region), type of medical specialty, underlying conditions, central venous catheters (CVCs) and bladder catheters in place, cultures taken from these catheters, and the outcome (I, III, IV). Nosocomial versus community acquisition of infection (I,III) was individually assessed in each case according

to criteria proposed by the CDC [51]. Immunosuppressive status was defined as cytotoxic therapy, total body irradiation no more than three months prior the onset of candidemia, or systemic cortisone (at least 40mg per day at the onset of the cortisone treatment) no more than one month prior the onset of candidemia (I, III).

The number of blood samples for culture in HUCH between 1989–1992 was 40,079 (range per year, 9,430–10,918), and between 2000-2004 was 95,341 (range per year, 17,745–20,063); data were unavailable between 1993–1999. The mean number of blood cultures per 10,000 patient days increased by 70% from 200 during 1989–1992 (range by year, 200-210) to 340 during 2000-2004 (range by year, 320–370) (III).

The annual numbers of patient days and discharges were acquired from the HUCH administration (I, III).

4.3 Surveillance methodology for bloodstream infections of the Finnish Hospital Infection Program (III)

The Finnish Hospital Infection Program (SIRO) was established at the end of 1997. Two surveillance modules have been executed since 1999, including hospital-wide surveillance for nosocomial BSIs. Surveillance is active, prospective, and hospital-wide, covering all patients admitted to departments offering acute care. Blood samples are collected according to clinical decision, and no routine surveillance cultures are performed on asymptomatic patients. Candidemia data for the HUCH during 1999-2004 were obtained from SIRO. Local infection control nurses recorded clinical and microbiological data on a standardized case-record form sent monthly to the National Public Institute of Health and Welfare (THL) and entered into a common database. An episode of candidemia was defined as a patient with at least one blood culture that tested positive for *Candida* spp., notifications of the same species within one week after the first diagnostic sample in the same patient were also defined as one episode. The nosocomial acquisition of infection was defined according to criteria proposed by the CDC [51]. Data collected with each notification included each patient's unique national identity number, age, sex, date of admission, date of specimen, specialty, causative micro-organisms, and their sensitivity to antimicrobial agents. Of the underlying conditions and possible risk factors, type of delivery, whether the patient was newborn, whether a hematological or solid malignancy was present, and whether the patient had undergone organ transplantation or hemodialysis were recorded, as was the presence of CVC, prior surgery and treatment in an intensive care unit (ICU). The outcome at 7 and 28 days from the date of the first positive blood culture for each patient was obtained from the National Population Information System by the use of unique person identifiers.

4.4 Fluconazole consumption and survey on prophylaxis policy (II–III)

The data on total fluconazole consumption as defined daily doses (DDDs) per 1,000 inhabitants per year during 2000–2007 in Finland were acquired from the National Agency of Medicine (II). Data on fluconazole consumption as grams per year (intravenous and per oral) in HUCH, available during the periods 1991–1999 and 2003–2004, were obtained from the hospital pharmacy (III).

Infectious diseases specialists in the five tertiary care hospitals were interviewed by telephone with a structured questionnaire about written guidelines or routine practices for fluconazole prophylaxis in their hospital among the following patient groups (adults (≥15 years) and children (<15 years) separately): patients with hematological or other malignancy, patients treated in ICU, surgical patients, organ transplant patients and premature neonates (III).

4.5 Description of microbiological methods (I–IV)

Nationwide studies (I–II)

Detection and species identification of *Candida* isolates were performed in the notifying laboratories according to protocols in use in each laboratory.

Candidemias in HUCH and the Helsinki-Uusimaa region (I, III–IV)

The blood specimen collection method included obtaining blood in two pairs of aerobic and anaerobic bottles (approximately 10ml and 5ml per bottle for adults and children, respectively), from two different sites according to international guidelines. Yeasts in circulation were detected with the automated blood culturing system (Bactec™; BD, Franklin Lakes, NJ, USA; or Bact/ALERT™, Marcy l'Étoile, France), and the organisms were identified according to standard procedures. Blood culture bottles in which yeast cells were observed with gram stain were subcultured on Sabouraud agar plates. Primary identification of *C. albicans* was made with a positive germ tube test, and the result was confirmed by the detection of chlamydospores on cornmeal-Tween agar plates. If the germ tube tested negative or if no chlamydospores could be detected, the yeast strain was identified by fermentation tests (in-house prepared tube tests), and from July 1997 on by commercial assimilation tests (API20C or ID 32 C; BioMerieux, France).

C. albicans molecular typing (IV)

Blood culture isolates were stored at -70°C in skimmed milk glycerol tubes (prepared in house) in the microbiology laboratory. In case of multiple sampling from the same

patient, as a rule only the first isolate was stored. Re-identification of the revived strains was performed according to the standard protocol in use.

Molecular typing by restriction enzyme analysis (REA) was performed in the National Public Health Institute (formerly KTL, currently THL). A single colony of *C. albicans* was selected from a Luria agar plate and grown overnight at 37°C in 3 ml of YEPD broth (0.3% yeast extract, 1% peptone, 2% glucose) pelleted by centrifugation and washed with 1 M sorbitol. The cells were then resuspended in 100 ml of 1 M sorbitol50 mM KH2PO4 with 3 ml of mercaptoethanol and 20 ml of Zymolyase 20T (20 mg/ml in 1 M sorbitol50 mM KH2PO4, ICN Immunochemicals, USA). The mixture was incubated at 37°C for 2 h. Spheroplasts thus prepared were lysed by the addition of 0.5 ml of GES buffer (30 g guanidium thiocyanate dissolved in 10 ml 0.5 M EDTA, pH 8 and 10 ml distilled water followed by the addition of 2.5 ml of 10% W/vol sarcosyl; the total volume is added to 50 ml with distilled water). After 20 min incubation at room temperature, 100 ml of 5 M potassium acetate was added, followed by incubation on ice for 20 min and the addition of 0.5 ml of chloroform-isoamylalcohol (24:1). The upper phase was separated into a new tube after centrifugation. The DNA was then precipitated with 0.5 ml of isopropanol and washed with 70% ethanol. The DNA was dissolved and stored in TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) at 4°C until digestion with restriction endonucleases EcoRI or MspI for 24 h at 37°C under conditions recommended by the manufacturer (Boehringer Mannheim, Mannheim, Germany). Immediately thereafter, the DNA fragments were separated electrophoretically on 0.7% agarose gels at 20 V for 20 h. The gels were stained with ethidium bromide and photographed. DNA digests of 12 isolates were run in each gel. For control, a lambda EcoR1/HindIII DNA marker (Boehringer Mannheim, Mannheim, Germany) was run in the first and last lane of each gel. REA patterns were examined blindly by two of the investigators (JV–V, ES) using direct visual comparison of the patterns with the molecular weight standard. All samples showing initially similar REA patterns were reanalyzed and confirmed through side-by-side comparison. A difference of more than one band in the REA pattern was considered as an indication of genetic difference between strains.

4.6 Calculation of incidence and mortality rates and statistical analysis (I–III)

Denominators to calculate age- and sex-specific incidence rates as well as mortality rates were acquired from the National Population Information System during the periods 1995–1999 (I) and 2004–2007 (II). The average annual incidences were calculated by using the total number of cases and population during the study periods. To evaluate secular trends, rates of candidemia cases in different age and sex groups were calculated for each 6-month period (I) and 12-month period (II). Using each patient's unique national identity number for register linkage, individual death

data were acquired for the period 2004–2007 from the same register to determine survival at 28 days from the first positive blood culture. The Poisson regression model served to assess whether the observed changes in the incidence rates and fluconazole consumption were statistically significant. Data were analyzed with Epi-Info version 6.04 (CDC, Atlanta, Georgia, USA) and SPSS for Windows version 11 (Chicago, USA) (I-III). Categorical variables were analyzed with the $\chi 2$ test using Yates`s correction, or Fisher's exact test as appropriate. Continuous variables were analyzed using the Student's t test or by the Mann-Whitney U test, depending on the sample distribution. To compare the case fatality (%) and the proportion of *Candida* spp. in HUCH over time, the 18-year study period was divided into three subperiods: 1987–1992, 1993–1998 and 1999–2004 (III). To assess whether there was a time trend in the proportion of different *Candida* species, a generalized linear model with log link was fitted to the data (III).

4.7 Ethical aspects

The Ministry of Social Affairs and Health as well as the Ministry of Education and Culture approved the review of the patients' charts (I, III, IV). The Finnish Data protection Authority approved the research use of the SIRO data (III). THL authorized the use of NIDR data and the National Agency for Medicines permitted the use of the data on fluconazole consumption (I–II).

5 Results

5.1 Incidence of candidemia (I–III)

5.1.1 Incidence in Finland (I–II)

The total number of candidemia cases reported to NIDR was 479 during 1995–1999 and 603 during 2004–2007. The median age of the patients was 59 and 64 years (range 0–94 years), respectively, with male dominance (60–56%).

The annual incidences according to age- and sex-specific groups appear in Table 8. The average annual incidence of candidemia increased between the two study periods from 1.9 to 2.9 per 100,000 population (during 1995–1999 and 2004–2007, respectively). The annual incidence increased from 1.7 per 100,000 population in 1995 to 2.2 in 1999, but during 2004–2007 varied from 2.6 to 3.1 with no trend. According to the five tertiary care catchment areas, the average incidence varied between 1.3–2.2 and 2.4–3.9 during 1995–1999 and 2004–2007, respectively.

During 1995–1999, the average annual incidence of candidemia was highest at 9.4 per 100,000 population among infants <1 year of age, although this age group constituted only 6% of all candidemia cases. During 2004–2007, however, the average annual rate in infants <1 year of age was lower. By age and sex, the incidence rates increased most between study periods in patients >65 years of age, from 5.2 to 8.8 per 100,000 population (during 1995–1999 and 2004–2007, respectively), particularly in males. The highest annual incidence, 27.3 per 100,000 population, was observed in male infants <1 year of age during 1999, while the incidence was lowest in patients 1–15 years of age during both study periods. In males aged 16–65 years, the incidence rose significantly during 1995-1999, from 1.0 to 2.4 per 100,000 population ($P<0.05$ by Poisson regression), but during 2004–2007 the increase was non-significant. In all age groups the average annual incidence was higher in males than in females during both study periods.

TABLE 8. Annual incidences of candidemia by age- and sex-specific groups in Finland, 1995–1999 and 2004–2007.

Sex	Rate[a]										
	Age group (y)	1995	1996	1997	1998	1999	1995-1999	2004	2005	2006	2007	2004-2007
Male	<1	12.5	10.0	3.3	6.9	27.3	11.9 (18)	6.8	13.6	3.3	13.3	9.2 (11)
	1–15	1.2	0.4	0.6	0	0.2	0.5 (12)	0	0	0.2	0	0.1 (1)
	16–65	1.0	1.9	1.9	2.1	2.4	1.8 (159)	2.1	2.3	2.5	2.7	2.4 (169)
	>65	7.7	6.3	6.1	9.4	7.7	7.4 (97)	13.1	10.2	12.5	13.2	12.2 (156)
	All	1.9	2.1	2.1	2.5	2.8	2.3 (286)	3.1	3.0	3.4	3.7	3.3 (337)
Female	<1	6.5	3.4	0	3.6	21.3	6.9 (10)	3.6	3.5	10.4	0	4.4 (5)
	1–15	0.6	0.4	0.6	0.2	0.6	0.5 (12)	0.2	0.2	0.7	0.7	0.5 (8)
	16–65	1.0	0.8	1.1	1.2	0.8	1.0 (84)	1.8	1.7	1.8	2.0	1.8 (126)
	>65	4.2	4.3	3.4	3.6	4.2	3.9 (87)	7.4	6.0	6.4	6.4	6.6 (127)
	All	1.5	1.4	1.4	1.5	1.6	1.5 (193)	2.5	2.2	2.6	2.6	2.5 (266)
All	<1	9.6	6.6	1.7	5.3	24.4	9.4 (28)	5.2	8.7	6.8	6.8	6.9 (16)
	1–15	1.0	0.5	0.6	0.1	0.4	0.5 (24)	0.1	0.1	0.4	0.3	0.3 (9)
	16–65	1.0	1.4	1.5	1.7	1.6	1.4 (243)	2.0	2.0	2.2	2.3	2.1 (295)
	>65	5.4	5.0	4.4	5.7	5.6	5.2 (184)	9.7	7.7	8.8	9.1	8.8 (283)
	All	1.7	1.7	1.8	2.0	2.2	1.9 (479)	2.8	2.6	3.0	3.1	2.9 (603)

[a]Rate per 100,000 population (number of cases).

5.1.2 Incidence in HUCH (I, III)

A total of 364 episodes of candidemia were observed during 1987–2004 (range by year, 12–32) in 358 patients. Most (96%) of the infections became evident >2 days after hospital admission; 4% were related to a preceding hospital stay.

Incidences of candidemia by species appear in Figure 1. The average annual incidence varied from 0.41 per 10,000 patient-days during 1987–1992 to 0.37 during 1993–1998 and to 0.44 during 1999-2004 (range by year, 0.26–0.59); no increasing trend was detected in annual incidence. The annual incidence of *C. glabrata* for the entire study period increased (P<0.01), while the incidences of *C. albicans* or non-*C. albicans* spp. as a whole showed no statistically significant trend.

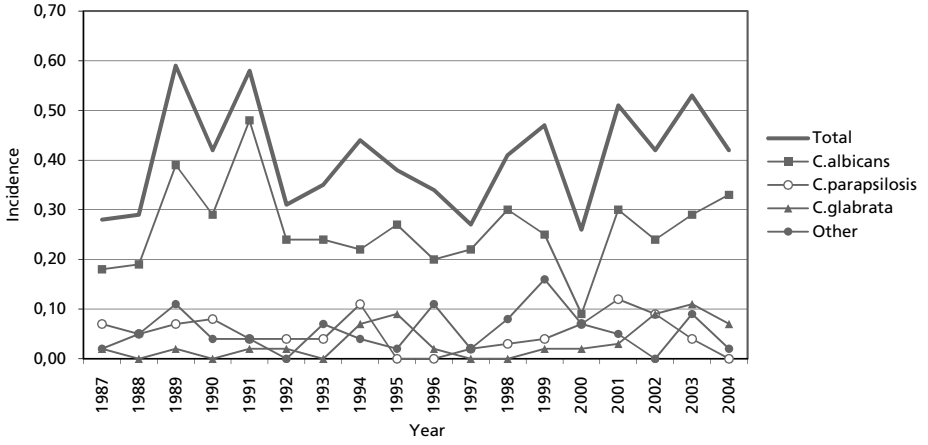

FIGURE 1. Candidemia incidence per 10,000 patient days at the Helsinki University Central Hospital, 1987–2004.

5.2 Patient characteristics and underlying conditions in HUCH (I, III)

The median age of the patients with candidemia was 50 years (range, <1–89 years) and 59% were male; no changes were detected in age and gender distribution during 1987–2004. The proportion of patients treated in the ICU increased from 24–27% during 1987–1998 to 44% in 1999–2004 (P<0.05). This increase was related to both surgical patients and newborns representing half of ICU patients. Overall, the proportion of surgical patients increased from 43–46% during 1987–1998 to 55% during 1999–2004, but this increase did not reach statistical significance (P=0.05). The proportion of hematological patients decreased significantly from 21–27% to 11%, and patients with solid malignancies from 24–25% to 9% by 1999–2004 (P<0.05). Organ transplantation (solid organ or bone marrow transplant) preceded

candidemia in only 2–5% of the patients. Information on a CVC in place was available for 347 episodes (95%): a catheter was present in 77–78% of those episodes during 1987–1998 and in 72% during 1999–2004. None of the cases had human immunodeficiency virus (HIV) infection.

A summary of patient characteristics and predisposing factors available only from chart review in HUCH during 1987–1998 appears in Table 9. At the onset of candidemia, all cases had at least one predisposing factor. The main predisposing factors were immunosuppressive state or leucopenia and gastrointestinal surgery. Biopsy-proven deep *Candida* infection was rare.

TABLE 9. Patient characteristics and predisposing factors in patients with nosocomial candidemia in HUCH, 1987–1998.

Characteristic or predisposing factor*	Number of patients, (%)	
	1987–1992 N = 128	1993–1998 N = 96
Duration of hospital stay prior onset of candidemia	21 days (range 0–165)	17 days (range 0–435)
Bladder catheter	44 (34)	37 (38)
Immunosuppressive state**	58 (45)	44 (46)
Leucopenia***	26 (20)	23 (24)
Preceding gastrointestinal surgery	50 (39)	28 (29)
Severe trauma	6 (5)	6 (6)
Biopsy-proven deep Candida infection	6 (5)	12 (9)

* one patient can have several predisposing factors; ** defined as cytotoxic therapy or total body irradiation ≤3 months before onset of candidemia or systemic cortisone (dose ≥40 mg daily at onset of cortisone treatment) ≤1 month before onset of candidemia; *** leucocytes ≤1 x 10E9/l and/or neutrophils ≤0.5 x 10E9/l.

5.3 Causative Candida species (I–III)

Distribution of *Candida* species in Finland (I–II)

The most frequent *Candid*a spp. encountered was *C. albicans* contributing to 335 (70%) cases during 1995–1999 and 406 (67%) cases during 2004–2007 (Figures 2 and 3). The proportion of non-*C. albicans* spp. as a whole remained constant in Finland. The most common of these, *C. glabrata*, increased in proportion from 9% in 1995–1999 to 19% in 2004–2007, but its proportion did not change during either of the study periods. *C. krusei* decreased in proportion from 8% to 3%, while *C. parapsilosis* remained stable at 5% between both periods of observation. The other species identified were *C. pelliculosa* (5 cases), *C. guilliermondii* (5 cases), *C. lusitaniae* (4 cases), *C. dubliniensis* (3 cases), and *C. rugosa* (1 case). The causative *Candida* spp. was unknown in 14 cases (3%) during 1995–1999 and in 4 cases (1%) during 2004–2007, respectively. Two *Candida* species were reported in 7 (1%) cases during 2004–2007.

According to the five tertiary care districts in Finland, the proportion of species other than *C. albicans* peaked during 2004–2005 in Turku at 44–42% and in 2004 in Tampere at 42%, consisting mainly of both *C. glabrata* and *C. parapsilosis*.

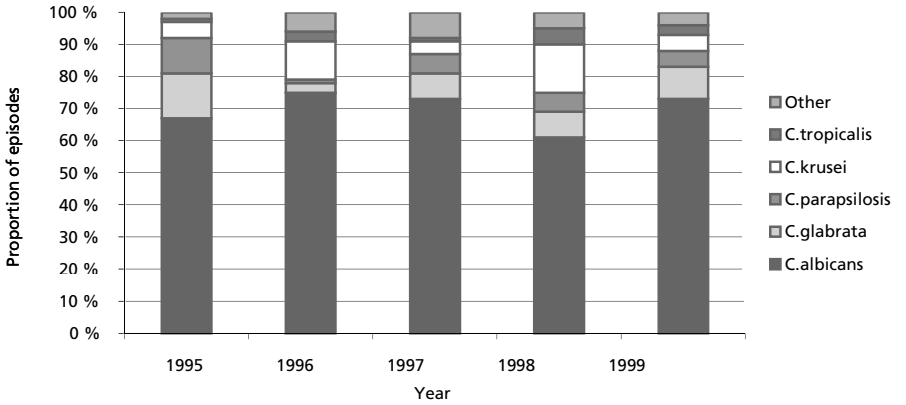

FIGURE 2. Distribution of Candida species in Finland, 1995–1999.

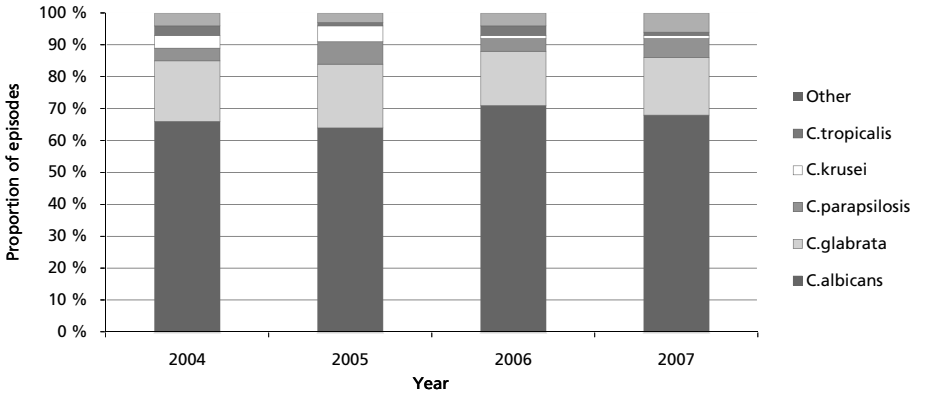

FIGURE 3. Distribution of Candida species in Finland, 2004–2005.

Distribution of *Candida* species in HUCH (I, III)

A total of 408 isolates were recovered from samples obtained during 364 candidemia episodes in HUCH during 1987–2004. Polymicrobial infections accounted for 11% (41) of the episodes; two *Candida* spp. were recovered from three episodes. The species of *Candida* was unknown in 1% of the episodes during 1987–1998 and in 5% during 1999–2004. *C. albicans* (65%) ranked the most common causative species for the entire study period, followed by *C. parapsilosis* (13%), *C. glabrata* (9%), *C. krusei* (5%), and *C. tropicalis* (2%). The proportion of *C. albicans* decreased from 71% (range by year, 65–82%) to 67% (range by year, 50–83%) between the subperiods 1987–1992 and 1993–1998, and was 58% (range by year, 36–78%) during 1999–2004. The

proportion of *C. albicans* decreased significantly from 69% to 58% between 1987–1998 and 1999-2004, respectively (P<0.05), while no statistically significant trend was detected between the subperiods for non-*C. albicans* species as a whole. The total number of episodes caused by *C. glabrata* was 31, 19 of which occurred during 1999–2004; the proportion of *C. glabrata* increased from 3% during 1987–1992 to 14% by 1999–2004 (P<0.01), but no other significant trend in species distribution was detected.

5.4 Fluconazole consumption and prophylaxis policies (II–III)

Total fluconazole consumption in Finland and in the five tertary care districts appears in Figure 4. Total consumption increased significantly (P<0.01) from 19.57 DDDs per 100,000 population in 2000 to 25.09 in 2007 in all five regions. Per oral consumption comprised 89% (range by year, 88-90%) of total fluconazole use; the rest was parenteral use (II).

In HUCH, the data on fluconazole use were available for periods 1991–1999 and 2003–2004. Fluconazole use increased more than six-fold from 22 grams per 10,000 patient days in 1991 to 145 grams in 2004. Per oral use increased from 6 to 79 grams per 10,000, and intra venous use from 15.5 to 66 grams per 10,000 patient days by 2004 (III).

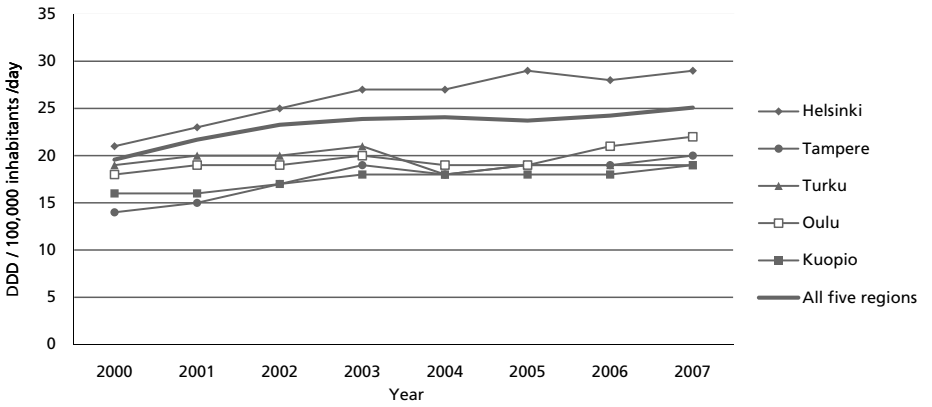

FIGURE 4. Use of fluconazole in daily defined doses (DDD) by tertiary care center districts in Finland, 2000–2007.

Prophylactic policies in Finnish tertiary care centers (II)

Fluconazole prophylaxis was used in all five tertiary care centers for adult acute leukemia patients treated with intensive chemotherapy over each period of

neutropenia, mostly since 2000, and in one center since 2007 (Table 10). In addition, in most centers, pediatric patients with acute leukemia received fluconazole prophylaxis during neutropenia. Fluconazole prophylaxis was administered to adult stem cell recipients in only one center, unlike pediatric stem cell recipients, who received fluconazole prophylaxis in most centers. Solid organ transplantations were performed in only one tertiary care center, and since 1998, only liver transplant recipients (adults and children) received fluconazole prophylaxis beginning immediately prior to or during surgery until at least 5 days postoperatively. In four of the five tertiary care centers, premature infants received fluconazole prophylaxis for two to six weeks or during invasive monitoring. All four centers have used prophylaxis since at least 2002–2004.

No tertiary care center used fluconazole prophylaxis in adult or pediatric patients with solid malignancies, intensive care patients or surgical patients.

TABLE 10. Fluconazole prophylaxis policies in the five tertiary care centers according to patient populations at risk.

Patient group	Prophylaxis policy
Adult patients	
Hematological patients	
Acute leukemia during neutropenic phase	Routinely in four centers since 2000, in one center since 2007. Written guidelines in three centers.
Stem cell recipients during neutropenic phase	Routinely in one center, written guidelines since 2007.
Patients with malignancies, ICU* patients and surgical patients	No routine prophylaxis.
Liver transplant recipients ≥5 days postoperatively**	Routinely since 1998, no written guidelines.
Other solid organ transplant recipients**	No routine prophylaxis.
Pediatric patients	
Hematological patients	
Acute myeloid leukemia during neutropenic phase	Routinely in four centers since 1998-2000. Written guidelines in three centers.
Acute lymphoid leukemia during neutropenic phase	Routinely in two centers. Written guidelines in one center.
Allogeneic transplant recipients during hospital stay***	Routinely in three centers since 2000. Written guidelines in three centers.
Autologous transplant recipients during hospital stay or neutropenic phase	Routinely in four centers since 2000. Written guidelines in two centers.
Premature neonates 2–6 weeks or during invasive monitoring	Routinely in four centers since 2002-2004. Written guidelines in two centers.
Patients with malignancies, ICU patients, and surgical patients	No routine prophylaxis.
Liver transplant recipients ≥5 days postoperatively**	Routinely since 1998, no written guidelines.
Other solid organ transplant recipients**	No routine prophylaxis.

* ICU = Intensive care unit.
** Solid organ transplantations are performed in only one center.
*** Allogeneic stem cell stransplantations are performed in only three centers.

5.5 Patient outcome (I–III)

Outcome in population-based study in Finland during 2004–2007 (II)

Data on outcome were available for 598 (99%) case-patients during 2004–2007, 208 (35%) of whom died within one month of the first blood culture that tested positive for *Candida*. The case-patients who died were significantly older than those who survived (median age, 70 vs. 62 years; P<0.01). The one-month case fatality varied by year between 32–38% and by tertiary care region between 31–37%. The average annual mortality rate was 0.99 deaths per 100,000 population (range by year, 0.89–1.08; range by district, 0.91–1.27).

Oucome in HUCH (I, III)

During 1987–2004, 113 (31%) candidemia patients died within one month of the onset of candidemia. Patients who died were significantly older than those who survived (median age, 58 vs.45 years; P<0.01), more likely to have had candidemia caused by *C. albicans* than other species (77% vs. 60%; P<0.01), or to have had hematological malignancy (27% vs. 15%; P<0.05), and less likely to have stayed in an ICU (25% vs. 36%; P<0.05). The overall proportion of one-month case fatality remained stable over the three study periods (range per period, 30–33%). Among the different patient groups, case fatality increased in patients with hematological or solid malignancies, but not in newborns, patients with previous surgery or intensive care patients (Table 11). Of the 14 patients with organ transplants, 7 (50%) died within one month.

TABLE 11. Case fatality within one month of the onset of candidemia according to underlying conditions and risk factors in Helsinki University Central Hospital during 1987-2004

Underlying condition or risk factor*	Number of deaths, %							
	1987–1992		1993–1998		1999–2004		Overall	
Preceding surgery N = 177	20	34%	13	32%	18	23%	51	29%
Stay in the ICU** N = 120	13	37%	3	13%	12	19%	28	23%
Hematological malignancy N = 69	10	37%	12	46%	8	50%	30	43%
Solid malignancy N = 68	8	26%	8	33%	5	38%	21	31%
Newborn status N = 62	5	26%	0		3	9%	8	13%
Organ transplantation N = 14	3	100%	0		4	67%	7	50%

* one patient may have several underlying conditions or risk factors; ** ICU=Intensive care unit.

5.6 Epidemiological typing of C. albicans (IV)

DNA types were highly dispersed among the study population as 118 distinct DNA types were identified among 142 *C. albicans* strains from 130 patients. As many as 13 DNA types (codes CA1–CA13) were observed more than once, and 105 DNA types (code CAx) were unique. Both restriction enzymes yielded similar results; neither was superior in discriminating between DNA types.

One patient yielded a DNA type CA3 and a subtype CA3.1 on consecutive blood cultures, and only one patient yielded two DNA types (CAx and CAx) from subsequent blood cultures taken four days apart. One patient had two separate episodes of candidemia with a time interval of 279 days, and the same DNA type CA6 was isolated once during the first episode and twice with a time interval of 33 days during the second episode of fungemia.

There was no evidence in the typing results, which suggests epidemic clustering of the patients. Because of hospital transfers, the data were also analyzed according to the unit in which the patient was hospitalized three days prior to the first positive blood culture for yeast, but no clusters were detected. The greatest number of *C. albicans* blood culture strains was from the unit for gastroenterologic surgery (n = 27) and from the Surgical Hospital (n = 28), which perform most of the solid organ transplantations in HUCH. In 3 patients of almost 30 in these units, the same subtype was observed, but these candidemia cases were distributed across several months and in different bed wards. Despite the abundant patient transfers between bed wards and units performing invasive and non-invasive diagnostic procedures in HUCH, only one subtype found in 2 of a total of 63 patients was shared between different services.

6 Discussion

This study characterizes the epidemiology of candidemia in Finland during two separate five-year periods of nationwide population-based surveillance. Overall, annual as well as age- and sex-specific incidence rates were calculated, along with the outcome of illness during the latter study period. In addition, fluconazole prophylaxis policies in all five tertiary care centers as well as fluconazole consumption in Finland were analyzed during 2004–2007. Over 18 years of observation, the incidence, predisposing factors, patient characteristics, and outcome of candidemia, as well as fluconazole consumption, were studied in the largest tertiary care center (TCC) in Finland. The *C. albicans* blood culture isolates from patients within a defined region in Finland were characterized with the molecular typing method. The main findings and limitations of this study are dicussed below.

6.1 Candidemia incidence rates

6.1.1 Candidemia incidence in Finland

Our nationwide population-based studies show that the incidence of candidemia in Finland is relatively low. During the period 1995–1999, however, we observed a consistent year-to-year increase, and the average annual incidence of candidemia increased between the periods 1995–1999 and 2004–2007 from 1.9 to 2.9 per 100,000 population, respectively. Instead, candidemia incidence in Finland remained stable during the years 2004–2007.

The rates we observed are only one third to one fourth of those reported from the USA, with most reports ranging from 6.0 to 10.0 per 100,000 population [23, 82–84] Two of these studies were based on sentinel surveillance implemented in selected urban areas, and thus they may not be representative of the general US population. Low incidence rates comparable to ours have been reported from Norway and Canada [11, 17], with somewhat higher reported from elsewhere in Europe [87, 89], and considerably higher rates (10.4 per 100,000 population) from Denmark [85, 162]. Extended surveillance periods have been reported from Iceland (1980–2006) and Norway (1991-2003), both with an increasing incidence over time [17, 86]. The increasing rate of candidemia we observed between periods in the 1990s and 2000s is in line with those of other population-based studies, thus highlighting the growing importance of bloodstream infections (BSIs) caused by *Candida* spp. among vulnerable hospitalized patients.

The constant male dominance we observed is consistent with most reports from the USA and Europe [17, 82, 83, 85], although not unequivocally [11, 23]. By age and sex, incidence rates increased in patients >65 years of age, particularly in males

(Table 8). Incidences comparable to ours in this age group have been observed in Norway and Spain [17, 89], while rates have been considerably higher in Denmark, Iceland, and the USA [83, 85, 86]. During the 1990s in Finland, the highest age-specific incidence rate was in infants <1year of age. Interestingly, in the two youngest age groups (<1 year-15 years of age), the incidence in Finland was lower during the 2000s than in the 1990s, partly due to the fading of an outbreak of *C. parapsilosis* in the neonatal intensive care unit (NICU) of the HUCH since 2002. Clearly higher incidence rates (20–75 per 100,000 population) in infants <1 year of age have been reported from North America and Spain [11, 83, 89], while incidences in other Nordic countries have been only somewhat higher than in Finland [11, 17, 83, 85, 86, 89].

The conspicuous differences in candidemia rates between countries may be real, but may also be due to differences in the representativeness and age distributions of the study populations as well as variations in patterns of health care delivery and clinical practices, including the frequency of using blood cultures in diagnostics, differences in antibiotic use patterns, and the resistance situation. In a previous study, *Candida* spp. represented only 4% of all pathogens in nosocomial BSIs in Finland, and the prevalence of antibiotic resistance among bacterial findings was lower than in the USA [64].

While our laboratory-based surveillance data of candidemia are representative of the entire population, the data on patient characteristics collected with each notification are limited, and the proportion of nosocomial vs. community-onset candidemias is unknown.

6.1.2 Candidemia incidence in HUCH

Our 18-year study in the largest tertiary care hospital in Finland showed a constant low incidence of candidemia between 0.26 and 0.59 per 10,000 patient days. This is surprising given the increase in high-risk activities for invasive fungal infections, such as solid organ and stem cell transplantations, which more than doubled during our study. Non-nosocomial candidemia was very rare in HUCH, as only 4% of candidemia cases were detected within two days of hospitalization, which contrasts sharply with reports from the USA, where one fifth of candidemias develop in patients before or on admission to the hospital [83, 258].

While several centers in Europe have reported incidence rates comparable to ours [5, 10], higher rates have been observed in Belgium, France, and Italy [16, 168, 172]. In addition, the rate we observed in HUCH is one fourth to one sixth of the rates reported from the USA [23, 127]. In-hospital incidence rates in the USA and Europe have been largely stable or increasing during 1990-2000s, but decreasing incidence has been reported from Belgium [172] and also from intensive care units (ICUs) in the USA during the 2000s [15]. However, a 3.5-fold increase was observed during 1998–2008 in Australia, particularly in adult TCCs [173]. The current overall

trends of candidemia incidence are not clear. The reported rates in Europe have been lower than in the USA, and while many centers have observed stable or even decreasing incidences, also considerable increases have been reported.

This considerable variation in rates in different centers highlights major differences in treatment practices, in vulnerable patient populations, and in diagnostic activity (e.g., the frequency with which blood cultures are used in diagnostics). Also, the manner in which the surveys are conducted (i.e., single-center vs. multicenter studies) may affect the results. While the number of patients at risk for *Candida* BSIs appears to be increasing, more of the care of these patients has shifted to non-ICU settings, and patients with indwelling central catheters are cared for at home. This change of practice may in part increase the rates of candidemia outside traditional high-risk areas [173]. Definitions of nosocomial infection may vary, as can determining whether an infection is related to previous hospitalizations [258]. During our study, the number of blood cultures per patient day increased markedly; thus, the stable incidence we observed is not explained by low diagnostic activity.

Our study has several limitations. The number of candidemias during 1987–1998 was acquired from microbiology laboratory logbooks, and during 1999–2004, from the Finnish Hospital Infection Program (SIRO) (this different data source may affect our observed rates). The data on blood samples taken for culture covers only part of our study period, although with a consistently rising trend.

6.2 Patient characteristics in HUCH

The proportion of ICU patients increased significantly during the study period and became one of the major patient groups at risk. The proportion of surgical patients increased, while those of traditional risk groups (i.e., patients with hematological or solid malignancy) declined.

Our study confirms the importance of surgery (particularly gastroenterology surgery), treatment in the ICU, and hematological and solid malignancies as factors contributing to nosocomial candidemia. Several centers around the world have reported rising rates of candidemia among intensive care patients [5, 10, 92, 165, 172, 174, 183]. The high proportion of surgical patients we observed is in accordance with previous observations from Europe and Australia [10, 12, 173], but contrasts with a study from the USA in which surgical patients comprised 18% of candidemias [83]. The decline in the proportion of hematological patients in HUCH also parallels the implementation of fluconazole prophylaxis in this patient group.

None of the patients with candidemia had HIV infection, which reflects the low prevalence of HIV infection in Finland [259]. The proportion of patients with HIV-infection among candidemia patients has been high ranging from 10% to 15% in the USA [83, 84], while in Europe, these patients comprise 0.6–3% of candidemia cases [10, 12, 92].

The proportion of newborns among candidemia patients increased during 1999–2004 and has been associated with a prolonged outbreak in premature neonates that has subsided since 2002 [194]. Newborns (patients <1 year) have accounted for ~8% of candidemia patients in several centers in Europe [12, 92].

The patient data recorded in the SIRO are limited, and we did not review patient charts during the years 1999–2004. In addition, the data abstracted from patient charts have limitations: the information is not uniform, is sometimes hand-written, or is missing altogether. Due to our long observation period, and thus multiple changes in hospital departments, we could not analyze patient days according to specialty.

6.3 Causative Candida species

Our nationwide population-based study observed no changes in the proportion of *C. albicans* between the 1990s and 2000s in Finland, but the proportion of *C. glabrata* increased considerably from 9% to 19%, ranking second after *C. albicans* during the 2000s. Other *Candida* species were isolated only rarely, while the proportion of *C. krusei* decreased from 8% to 3% between our study periods. Accordingly, the predominating causative organism in HUCH during our 18 years of observation was consistently *C. albicans*; its proportion decreased significantly, however, while that of *C. glabrata* increased.

The stable predominance of *C. albicans* in Finland contrasts with several reports from the USA, Australia, and Europe, in which the proportion of non-*C. albicans* species has increased to 40-56% [14, 15, 22, 23, 65, 83, 85, 171, 173, 183], but is in line with nationwide studies from Iceland and Norway as well as with several reports from centers in Europe [5, 17, 86, 172, 182]. The precise pattern of causative species varies across countries and centers [22, 89, 173, 175], but some centers in the USA and Europe report that the incidence of *C. glabrata* has risen to second or third in rank [13, 15, 82, 260, 261]. The shift in causative *Candida* spp. remains still to be clarified, while the increasing proportion of *C. glabrata* has been associated with many factors including geographical variation, exposure to azoles or antifungal agents, differences in demographics and in patient comorbidites, and the ability of blood culture systems to isolate different *Candid*a species [15, 22, 262]. Interestingly, in HUCH the proportion of *C. glabrata* as a causative species rose only after 2001, while the consumption of fluconazole already began increasing through the 1990s. The cause of the decrease we observed in the proportion of *C. krusei* is unknown, but may be associated with patient demographics and underlying conditions.

Limitations to our study include a growing number of unknown *Candida* spp. (1% vs. 5 %, respectively) reported concomitantly with the shift in the use of data from SIRO. Changes in blood culture systems in HUCH over the years may also have contributed to changes in causative species.

6.4 Fluconazole consumption and prophylaxis policies in Finland

The consumption of fluconazole in HUCH increased six-fold during 1987–2004. The total consumption of fluconazole increased in all five regions in Finland during 2000–2007, which also suggests increased usage of fluconazole in tertiary care hospitals.

The consumption of fluconazole in HUCH is in the same range as that of a report from one TCC in Belgium during 1994–2004, but a multicenter study in Switzerland in 2000, and another in the USA in 1993, reported clearly lower consumption [5, 165, 182]. No association was observed between the use of fluconazole and distribution of *Candida* species causing BSIs in Switzerland or in Belgium [5, 182]. The role of fluconazole consumption in the shift of causative *Candida* species remains inconclusive, as several reports have observed the effect [13, 15, 165, 261], while others have not [5, 17, 22, 182].

Prophylaxis with fluconazole was used systematically during 2000s in patients with acute leukemias, liver transplant patients, and in premature infants in Finland. The prophylactic use of fluconazole in neonates and pediatric leukemia patients along with improved infection control practices may have contributed to the observed reduction in candidemia rates in patients <16 years of age. Despite the increase in fluconazole consumption in HUCH as well as in tertiary care center catchment regions in Finland, we observed no shift towards non-*C. albicans* species as a whole, but among them the proportion of *C. glabrata* increased.

Data on fluconazole usage in HUCH were available only for the years 1991–1999 and 2003–2004. Data on fluconazole consumption in HUCH, however, is general year-to-year use of fluconazole obtained from the hospital pharmacy with trends in the distribution of causative species, which is not generalisable to the individual patient level. Moreover, our nationwide survey is an ecological study in which we used data on the total consumption of fluconazole in different tertiary care catchment regions, in connection with our population-based data on incidence rates, to make inferences about individual patients based on average population statistics. In addition, with only laboratory-based reporting (NIDR), differentiating between nosocomial and community-onset infection is impossible. Consequently, we could not calculate fluconazole consumption as DDDs per patient-day and have no data on sensitivity to fluconazole. A further limitation of our study is that the guidelines and routine prophylaxis policies presented here are limited to patients in tertiary care centers, and thus affect candidemia rates in these hospitals and not in health-care institutions as a whole. Regarding infectious diseases, however, physicians of the centers play a key consulting role in their districts on policies affecting groups at high risk for candidemia. Furthermore, we did not inquire about the dosing of the prophylaxis, so the adequacy of the prophylaxis remains unknown.

6.5 Patient outcomes

The proportion of crude case fatality by one month remained stable at 30–33% in HUCH, and at 35%, remained stable in our nationwide study during 2004–2007 as well.

The case fatality rates we observed are in line with those of reports from the USA and most European countries [12, 23, 91, 92, 185], despite reports of clearly higher rates of 44–61% [9, 16, 89, 171, 174, 175, 183]. Patients who died were more likely to be old and to have hematological malignancy or candidemia caused by *C. albicans*, thus confirming the contribution of serious underlying diseases, treatments causing immunosuppression, and older age to the high case fatality rate. In HUCH, the low case fatality rate in patients treated in ICU reflects the fact that the majority of them were premature newborns with candidemia caused by *C. parapsilosis*. Patients with a previous organ transplant had the highest case fatality at 50%, but they were few (14).

A limitation of our study is that we had no data on deaths related directly to candidemia, and thus could not calculate attributable mortality, but only all-cause mortality.

6.6 Epidemiological survey of C. albicans

The 142 blood culture isolates of *C. albicans* from 130 patients were highly dispersed when analyzed using restriction-enzyme analysis (REA).

Our study covered a period of more than eight years and seven separate acute care hospitals, one of which was located at six different sites. This set-up should reduce the bias potentially associated with small patient materials from a single center where endemic subtypes could be prevalent. REA differentiated efficiently between *C. albicans* strains, and both restriction enzymes showed similar discriminatory power. Although a minority, among consecutive isolates from the same patient (11), REA demonstrated highly reproducible DNA patterns. No clusters or endemic subtypes were observed in the hospitals involved despite the abundant transfer of patients between them.

REA has been used to distinguish strains of *C. albicans*, although when compared with pulsed-field gel electrophoresis (PFGE) karyotyping, the results have been conflifting [45, 207, 263–266]. The application of these two methods in succession enhances differentiation between strains [263, 265, 267]. Using computer programs to analyze digitized REA patterns improves the accuracy of strain discrimination and the speed of analysis, while modern methods that use DNA amplification by PCR could offer accurate strain discrimination of both *C. albicans* and non-*C. albicans* spp. [102, 142, 264].

7 Conclusions and future considerations

The incidence of candidemia in Finland is globally relatively low, but did increase during the period 1995-1999, and between the two study periods in the 1990s and 2000s. In HUCH, the incidence of candidemia remained low and constant during 1987–2004, and consisted almost exclusively of nosocomial infections. *Candida* spp. are an important cause of nosocomial bloodsream infections in Finland, and continued surveillance is necessary to determine the overall trends and to reduce the impact of these infections in the future. Multicenter and nationwide studies contribute to a comprehensive data set across all susceptible patients groups and to the true influence of healthcare delivery on candidemias, while surveys performed in a hospital have tremendous value for therapeutic decision making and preventive measures at the local level. Surveillance to monitor the burden of disease and the emergence of resistance among the general patient population and among high risk patient populations should continue.

In nationwide studies, incidence rates increased in older age groups, particularly in males, while in patients in the younger age groups (<1 year-15 years of age), had lower incidence during the 2000s than in the 1990s in Finland. In HUCH, the study implied a significant shift in patient populations at risk, with surgical and intensive care patients gaining predominance. A future challenge is to uniformly recognize the adult high-risk patients treated in surgical wards and intensive care units as such and to apply them measures to prevent nosocomial infections and adequate antifungal prophylaxis.

The predominant causative species in Finland and in HUCH is *C. albicans*, but the proportion of *C. glabrata* increased considerably. While this increase stems partly from a change in blood culture systems and an older patient population (*C. glabrata* is rare in neonates), it may reflect a true shift in species causing candidemias. The increase in the proportion of *C. glabarata* has several implications both for microbiology laboratory (in identifying the clinical isolates in species-level and in antifungal susceptibility testing) and for clinical decicion making in choosing prophylactic, pre-emptive, and empirical treatments.

Fluconazole consumption increased not only in HUCH, but in all five tertiary care center regions in Finland as well. Fluconazole prophylaxis was systematically implemented in traditional high-risk patient groups (i.e., patients with acute leukemias, liver transplant patients, and in premature infants) and most probably contributed to the observed decrease of candidemia rates in these subpopulations. The role of antifungal prophylaxis with fluconazole in amplifying the shift in causative *Candida* species is inconclusive, but requires continued vigilance in the future.

The proportion of crude case fatality per one month was high, at 30–35%, and remained stable. This high case fatality ratio emphasizes the need for continuous surveillance to identify changes in predisposing factors to optimize preventive policies, including the use of antifungal prophylaxis, particularly in new risk groups. Given the substantial excess mortality due to candidemia and the difficulties encountered in administering early and effective antifungal therapy, better methods of prevention will decrease candidemia-associated mortality more than will advances in therapy.

Epidemiological typing with REA showed no clusters or endemic subtypes in the hospitals involved. Rather, as a rule, each patient was infected by a unique *C. albicans* strain, implying an endogenous source of candidemia. In the future, molecular methods for investigating suspected outbreaks caused by *Candida* spp. should be available in Finland, also.

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